



EXHIBIT 34

U.S. Patent No. 8,273,308 Infringement Chart

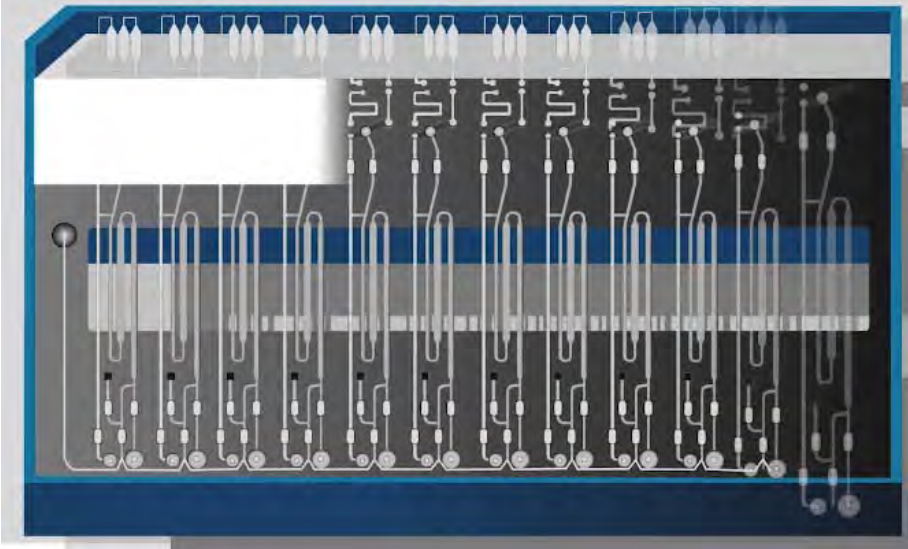
Claim	Claim Language	Infringement Evidence
1(a)	A system, comprising:	<p>To the extent the preamble is limiting, the accused instruments are a system.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p>  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited</p>

Claim	Claim Language	Infringement Evidence
		<p data-bbox="793 233 1136 266">May 31, 2019 (Exhibit 10)</p> <ul data-bbox="842 272 1892 342" style="list-style-type: none"> <li data-bbox="842 272 1892 342">• “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result.” <p data-bbox="793 380 1906 449"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul data-bbox="842 456 1913 1404" style="list-style-type: none"> <li data-bbox="842 456 1913 634">• “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” <li data-bbox="842 641 1913 1036">• “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. <li data-bbox="842 1042 1913 1263">• “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <li data-bbox="842 1269 1913 1404">• “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of

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		<p>the instrument with touchscreen computer, accessories, and reagents and consumables.”</p> <ul style="list-style-type: none"> • “NeuMoDx™ Molecular Systems are versatile; in addition to IVD tests, our system can also be used as an open system to process Laboratory Developed Tests (LDTs) that have been created and validated by your lab.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, last visited May 31, 2019, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> • “There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.”
1(b)	a microfluidic device	<p>The accused system comprises a microfluidic device.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

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		 <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 24, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from ‘sample to result’. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-

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		<p>sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.”</p> <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>0600101_Rev-D-IFU-NeuMoDx-RELEASE-Solution-US-ONLY-FINAL-25Oct2018.pdf (Exhibit 20)</p> <ul style="list-style-type: none"> • “NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE... The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) and the dried Master Mix contained in a NeuMoDx test strip. The System then dispenses the prepared RT-PCR-ready mixture into the NeuMoDx Cartridge where Real-Time PCR occurs.” <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> • “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.”

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		<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <p>“Patents”, http://www.neumodx.com/patents/, demonstrating that NeuMoDx marks its products with US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165 9,452,430; 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; 10,010,888; 9,382,532; 9,540,636; 9,499,896; 9,539,576; 9,637,775; and 10,093,963. (Exhibit 15)</p>

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		<div><h2>PATENTS</h2><table><tr><th>Product</th><th>Patents</th></tr><tr><td>CARTRIDGE</td><td>US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.</td></tr><tr><td>P02 (overall system and method)</td><td>US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.</td></tr><tr><td>EXTRACTION PLATE</td><td>US Patent Nos. 9,382,532; and 9,540,636.</td></tr><tr><td>XPCR MODULE</td><td>US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.</td></tr></table></div> <div><p>US10041062 (Exhibit 33)</p><ul style="list-style-type: none">Claim 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising: a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode; a nozzle of a liquid handling subsystem; an optical subsystem; a cartridge heater; a magnet vertically aligned with the magnet receiving slot; and an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein: the nozzle of the liquid handling system is coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample, the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge, the optical subsystem interfaces with a second portion of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and a third region of the cartridge is compressed between the cartridge heater and the cartridge platform.</div>	Product	Patents	CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.	P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.	EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.	XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.
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		<p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater.
1(c)	a computer-controlled heat source; and	<p>The accused system comprises a computer-controlled heat source.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieveing [sic] optimal operational and clinical performance for our customers and their patients.” “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary

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		<p>NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection

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		<p>chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating

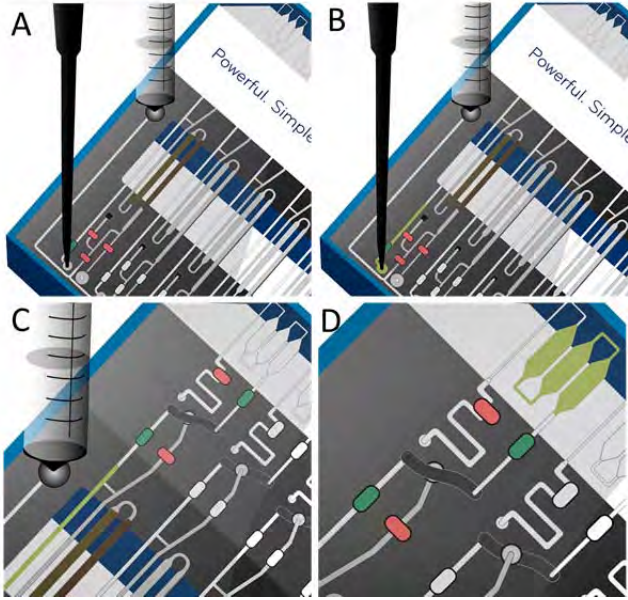
Claim	Claim Language	Infringement Evidence
		<p>layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:21-32 (“As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection 30 chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.”) • U.S. Patent No. 9,499,896 at 9:11-19 (“As shown in FIGS. 1, 4A-4B, and 7A-7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heater-sensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the system 100, a set of elastic elements 160

Claim	Claim Language	Infringement Evidence
		<p>configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 12:20-31 (“In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistance-to-voltage conversion circuit because the UT750 PID controller requires voltage as an input for PID control.”) U.S. Patent No. 9,499,896 at 11:63-12:4 “As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100.”
1(d)	a detector;	<p>The accused system comprises a detector.</p> <p><i>NeuMoDx™ Molecular Systems, NEUMODx,</i> http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems, NEUMODx,</i> http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of

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		<p>products of amplification.”</p> <p>JFO_2018-10-25_8009-Rev-B_NeuMoDx-96-Spec-Sheet (Exhibit 21)</p> <table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>NeuMoDx 288 Spec Sheet R2.pdf (Exhibit 22)</p> <table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>US10041062 (Exhibit 33)</p> <ul style="list-style-type: none"> Claim 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising: a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode; a nozzle of a liquid handling subsystem; an optical subsystem; a cartridge heater; a magnet vertically aligned with the magnet receiving slot; and an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein: the nozzle of the liquid handling system is 	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
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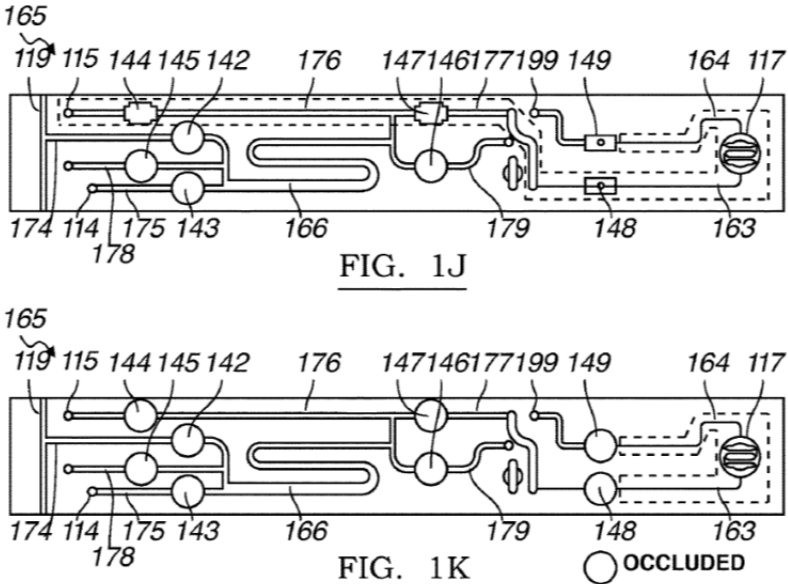
Claim	Claim Language	Infringement Evidence
		<p>coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample, the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge, the optical subsystem interfaces with a second portion of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and a third region of the cartridge is compressed between the cartridge heater and the cartridge platform.</p> <ul style="list-style-type: none"> Claim 8. The system of claim 1, wherein the optical subsystem comprises at least one unit including an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the biological sample, and to transmit emitted light from the biological sample, through the emission filter, and toward the photodetector. <p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater.

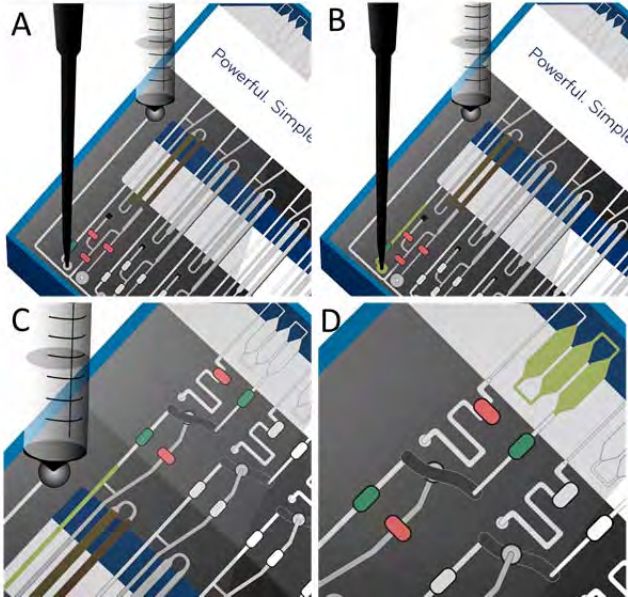
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> Claim 11. The system of claim 1, wherein the molecular diagnostic module further comprises an optical subsystem comprising a first unit and a second unit, wherein each of the first unit and the second unit includes a set of excitation filters, a set of emission filters, a set of photodetectors aligned with the set of emission filters, and a set of dichroic mirrors configured to reflect light from the set of excitation filters toward one of a set of nucleic acid-reagent mixtures at the cartridge, and to transmit emitted light from one of the set of nucleic acid-reagent mixtures, through at least one of the set of emission filters, and toward at least one of the set of photodetectors. Claim 12. The system of claim 11, wherein the molecular diagnostic module further includes a set of detection chamber heaters configured to heat a set of detection chambers through the second surface of the cartridge, and wherein the optical subsystem is configured to receive light, emitted from the set of nucleic acid-reagent mixtures at the set of detection chambers, from the first surface of the cartridge.
1(e)	wherein the microfluidic device comprises: an upstream channel;	<p>The accused system comprises a microfluidic device comprising an upstream channel.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

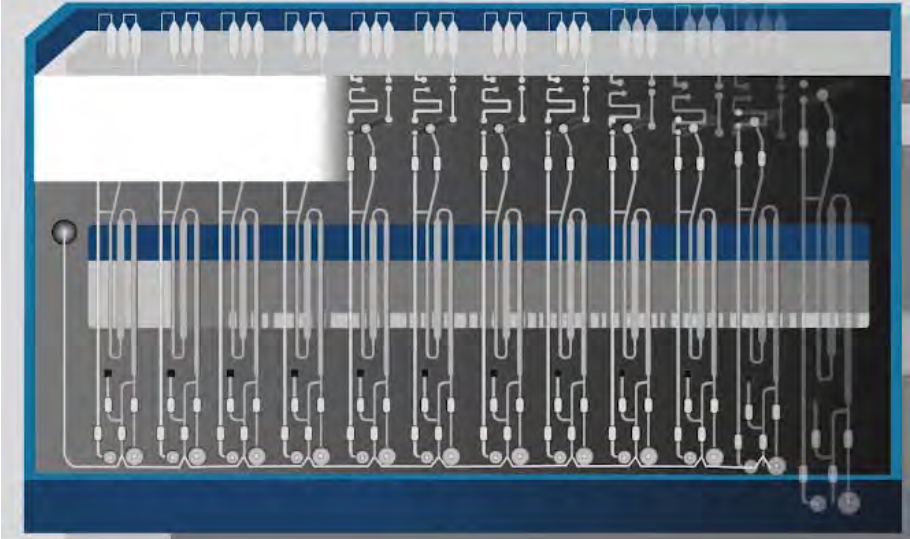
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 911 1115 943">US9738887 (Exhibit 31)</p> <ul data-bbox="842 951 1915 1390" style="list-style-type: none"> • Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent port, the fluid port, and the detection chamber.

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at Abstract (“A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region”) U.S. Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) U.S. Patent No. 9,738,887 at 15:31-35 (“The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.”) U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the

Claim	Claim Language	Infringement Evidence
		<p>sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at Figs. 1J and 1K:


Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1255 488 1381 521">FIG. 1J</p> <p data-bbox="1255 789 1381 821">FIG. 1K</p> <p data-bbox="1493 789 1661 821">○ OCCLUDED</p> <ul style="list-style-type: none"> <li data-bbox="846 854 1900 1032">• U.S. Patent No. 9,738,887 at 23:36-41 (“Each detection chamber 117 of the specific embodiment is identical and comprised of three interconnected channels, configured in a circular arrangement, with each of the interconnected channels approximately 0.4 mm deep and 1.6 mm wide at its widest point, resulting in a total volume of -10 mL for each detection chamber 117.”)
1(f)	[the microfluidic device comprises] a DNA manipulation module located downstream from the upstream channel;	<p data-bbox="793 1114 1894 1179">The accused system comprises a microfluidic device comprising a DNA manipulation module located downstream from the upstream channel.</p> <p data-bbox="793 1219 1900 1357"><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> <li data-bbox="846 1373 1900 1399">• “A series of microfluidic valves guides the PCR-ready solution through the

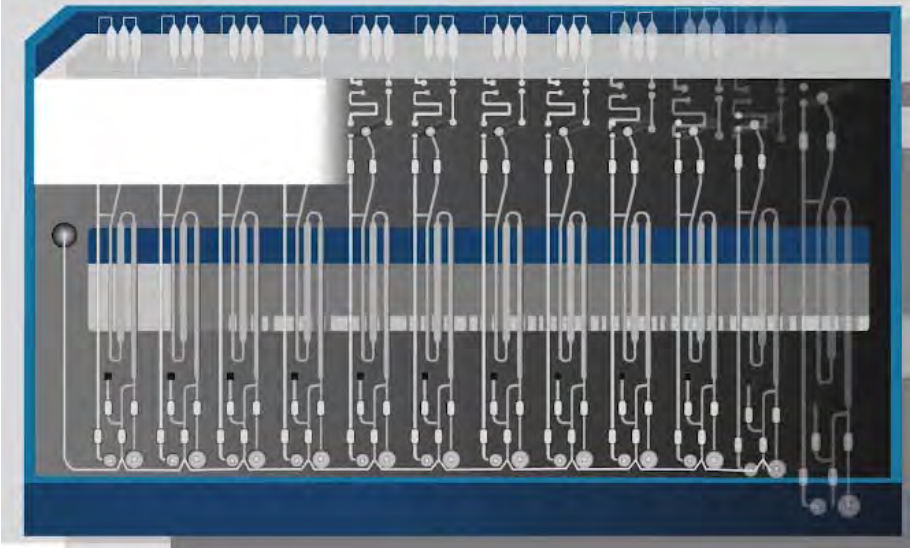
Claim	Claim Language	Infringement Evidence
		<p data-bbox="890 235 1816 302">cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08</p>  <p data-bbox="793 980 1877 1052"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="846 1062 1913 1240" style="list-style-type: none"> • “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.”

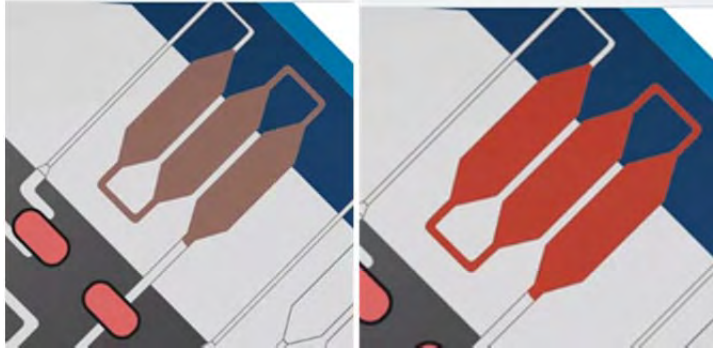
Claim	Claim Language	Infringement Evidence
		 <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 • U.S. Patent No. 9,738,887 at Abstract (“A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region.”)

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 2:36-3:5. (“As shown in FIGS. 1A-1C, an embodiment of a microfluidic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample port-reagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; and a set of fluidic pathways 160, each formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140.. In a specific application, the microfluidic cartridge 100 can be used to facilitate a PCR procedure for analysis of a sample containing nucleic acids.”) • U.S. Patent No. 9,738,887 at 13:7-18. (“The top layer 110 of an embodiment of the microfluidic cartridge 100 functions to accommodate elements involved in performing a molecular diagnostic procedure (e.g. PCR), such that a sample containing nucleic acids, passing through the cartridge, can be manipulated by the elements involved in performing the molecular diagnostic procedure. The top layer 110 is preferably composed of a structurally rigid/stiff material with low autofluorescence, such that the top layer 110 does not interfere with sample detection by fluorescence or chemiluminescence techniques, and an appropriate glass transition temperature and chemical compatibility for PCR or other amplification techniques.”) • U.S. Patent No. 9,738,887 at 13:35-42. (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • U.S. Patent No. 9,738,887 at 15:29-39 (“The segments may be arranged in at least one of several configurations to facilitate isolation, processing, and

Claim	Claim Language	Infringement Evidence
		<p>amplification of a nucleic acid sample ...”).</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 23:20-24 (“The top layer 110 of the specific embodiment of the microfluidic cartridge 100 functions preferably as described in Section 1.1, and is composed of polypropylene with low autofluorescence and a glass transition temperature suitable for PCR.”) • U.S. Patent No. 9,738,887 at 23:36-41 (“Each detection chamber 117 of the specific embodiment is identical and comprised of three interconnected channels, configured in a circular arrangement, with each of the interconnected channels approximately 0.4 mm deep and 1.6 mm wide at its widest point, resulting in a total volume of -10 mL for each detection chamber 117.”) • U.S. Patent No. 9,738,887 at 24:1-11 (“In the specific embodiment, the intermediate substrate 120 is composed of a polypropylene material to minimize cost and simplify assembly, and in the orientation shown in FIG. 11B, the top of the intermediate substrate 120 is 1.5 mm thick. The film layer 125, partially separating the intermediate substrate 120 from the top layer 110 is a polypropylene film with a nominal thickness of 50 microns. The film layer 125 is able to withstand temperatures of up to 95° C. encountered during fabrication and during an intended PCR procedure, while being thermally bondable to the top layer 110.”)
1(g)	[the microfluidic device comprises] a DNA manipulation zone within the DNA manipulation module and configured to perform PCR amplification of a sample;	<p>The accused system comprises a microfluidic device comprising a DNA manipulation zone within the DNA manipulation module and configured to perform PCR amplification of a sample.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that

Claim	Claim Language	Infringement Evidence
		<p>fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.”</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis,

Claim	Claim Language	Infringement Evidence
		<p>nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.”</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process

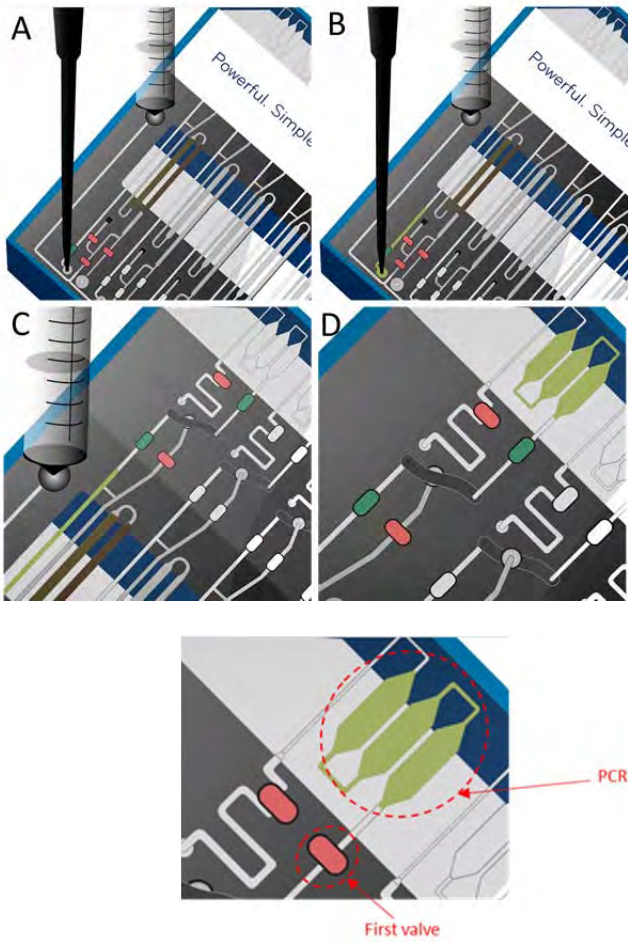
Claim	Claim Language	Infringement Evidence
		<p>begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p>  <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> • Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. • Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first

Claim	Claim Language	Infringement Evidence
		<p>sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <ul style="list-style-type: none"> • Claim 11. The cartridge of claim 10, further comprising 1) a heating region defined as a recessed region of the first layer that is parallel to the set of voids of the corrugated surface, and 2) a vent region, such that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber. <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample

Claim	Claim Language	Infringement Evidence
		<p>port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer.</p> <ul style="list-style-type: none"> • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple

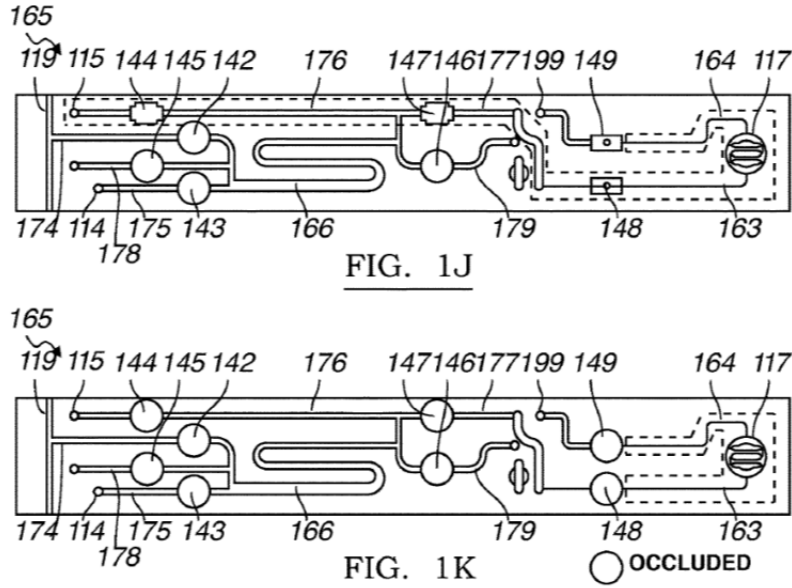
Claim	Claim Language	Infringement Evidence
		<p>wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter.</p> <ul style="list-style-type: none"> • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids.”) • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:49-65 (“The cartridge heater 153 functions to

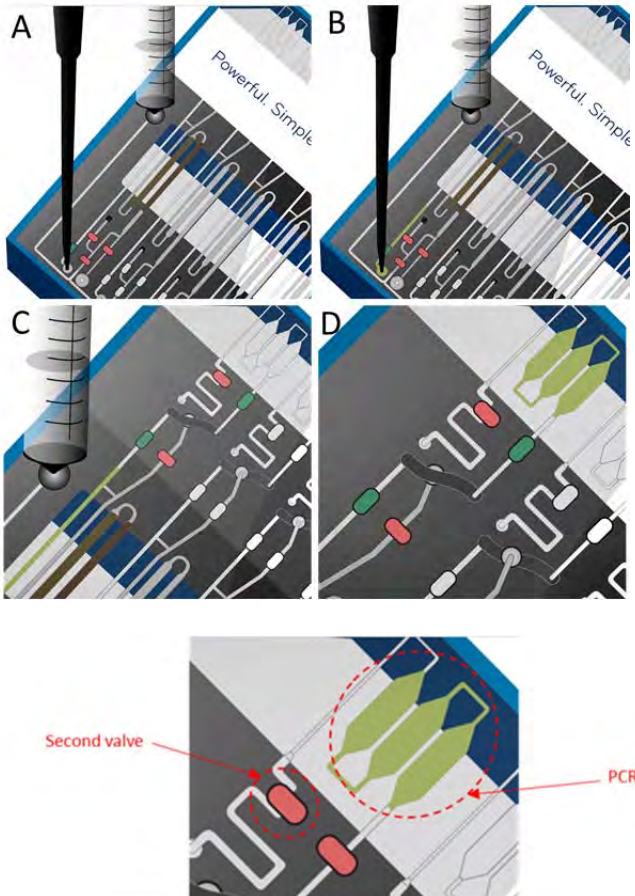
Claim	Claim Language	Infringement Evidence
		<p>transfer heat to a heating region 224 of a microfluidic cartridge 210, for inducing a pH shift to release bound nucleic acids from magnetic beads within the heating region 224... The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.")</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 29:44-47 (“In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.”)
1(h)	[the microfluidic device comprises] a first valve disposed within the DNA manipulation module upstream of the DNA manipulation zone;	<p>The accused system comprises a microfluidic device comprising a first valve disposed within the DNA manipulation module upstream of the DNA manipulation zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

Claim	Claim Language	Infringement Evidence
		 <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve

Claim	Claim Language	Infringement Evidence
		<p>guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as

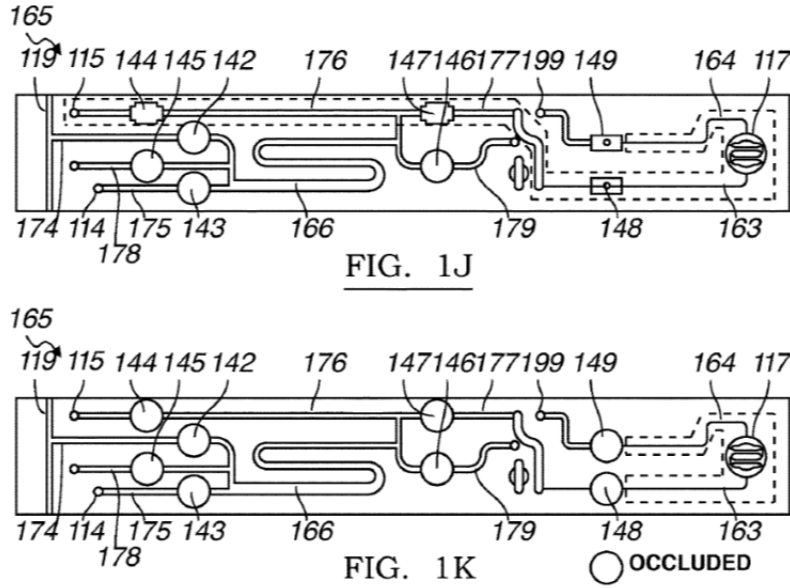
Claim	Claim Language	Infringement Evidence
		<p>shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)at Figs. 1J and 1K:</p>

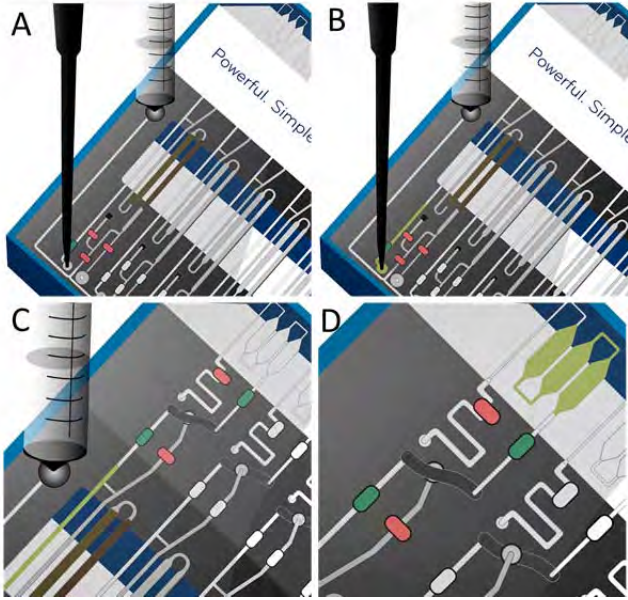
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1297 488 1430 521">FIG. 1J</p> <p data-bbox="1297 792 1430 824">FIG. 1K</p> <p data-bbox="1541 792 1703 824">○ OCCLUDED</p> <ul data-bbox="842 857 1913 1219" style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
1(i)	[the microfluidic device comprises] a second valve disposed within the DNA manipulation module	The accused system comprises a microfluidic device comprising a second valve disposed within the DNA manipulation module downstream of the DNA manipulation zone.

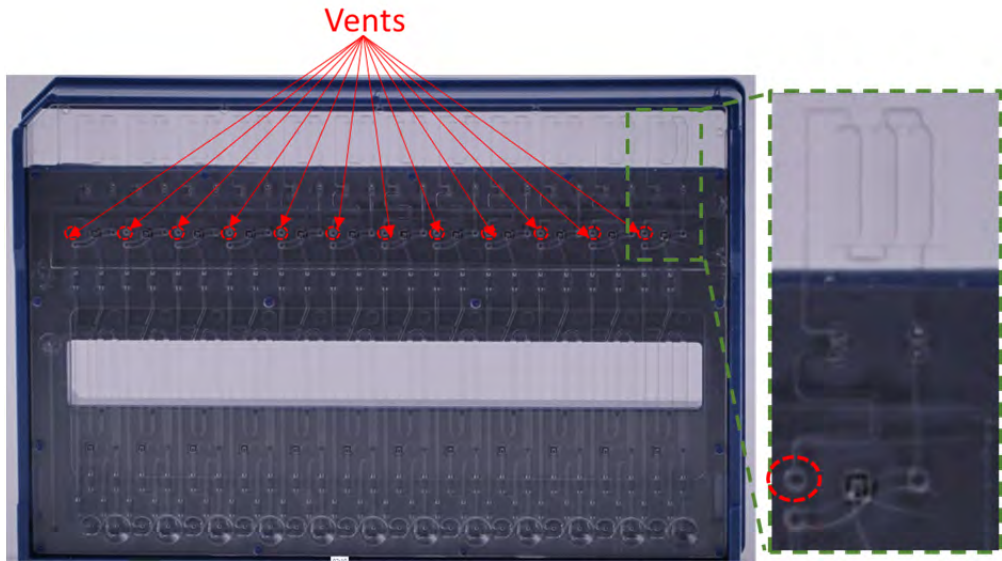
Claim	Claim Language	Infringement Evidence
	downstream of the DNA manipulation zone; and	<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 

Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second

Claim	Claim Language	Infringement Evidence
		<p>truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)at Figs. 1J and 1K:

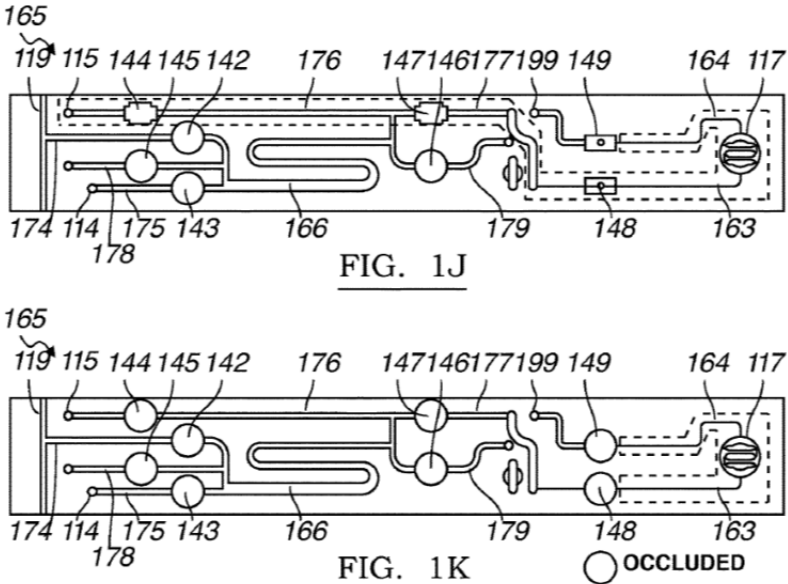
Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
1(j)	[the microfluidic device comprises] a vent disposed within the DNA manipulation module and separated from the upstream channel by the first and second valves.	The accused system comprises a microfluidic device comprising a vent disposed within the DNA manipulation module and separated from the upstream channel by the first and second valves.

Claim	Claim Language	Infringement Evidence
	upstream channel by the first and second valves;	<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>  <p>On information and belief, the accused cartridge comprises a vent disposed within the DNA manipulation module and separated from the upstream channel by the first and second valves.</p> <ul style="list-style-type: none"> • <i>Id.</i> at 2:10

Claim	Claim Language	Infringement Evidence
		 <p>The image shows a cartridge assembly with a series of vents along the top edge. Red lines point from the word 'Vents' to these vents. A dashed green box highlights a specific area on the right side of the cartridge, which is shown in a magnified view to the right. In this magnified view, a red dashed circle highlights a specific vent or opening.</p> <p>US9101930 (Exhibit 25)</p> <ul style="list-style-type: none"> Claim 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising: a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer; a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, are each at least partially separated from the corrugated surface of the intermediate substrate by an elastomeric layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate. Claim 11. The cartridge of claim 10, wherein the first layer is a unitary

Claim	Claim Language	Infringement Evidence
		<p>construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <ul style="list-style-type: none"> • Claim 13. The cartridge of claim 11, further comprising a heating region as a recessed region of the first layer that is parallel to the set of parallel voids of the corrugated surface, and a vent region, such that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber. • Claim 15. The cartridge of claim 13, wherein at least of the first fluidic pathway and the second fluidic pathway is coupled to an end vent configured to provide fine metering of fluid flow. <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent

Claim	Claim Language	Infringement Evidence
		<p>port, the fluid port, and the detection chamber.</p> <ul style="list-style-type: none"> • Claim 10. The cartridge of claim 1, wherein a terminal portion of the fluidic pathway is coupled to an end vent, configured to provide fine metering of fluid flow. • U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) • U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> U.S. Patent No. 8,738,887 at 15:4-6 (“A fluidic pathway 165 may also further comprise an end vent 199, which functions to prevent any fluid from escaping the microfluidic channel.”)
1(k)	a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the DNA manipulation zone when amplification of the sample occurs,	<p>The accused system comprises a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the DNA manipulation zone when amplification of the sample occurs.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” “The NeuMoDx™ Molecular Systems are a family of scalable platforms that

Claim	Claim Language	Infringement Evidence
		<p>fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

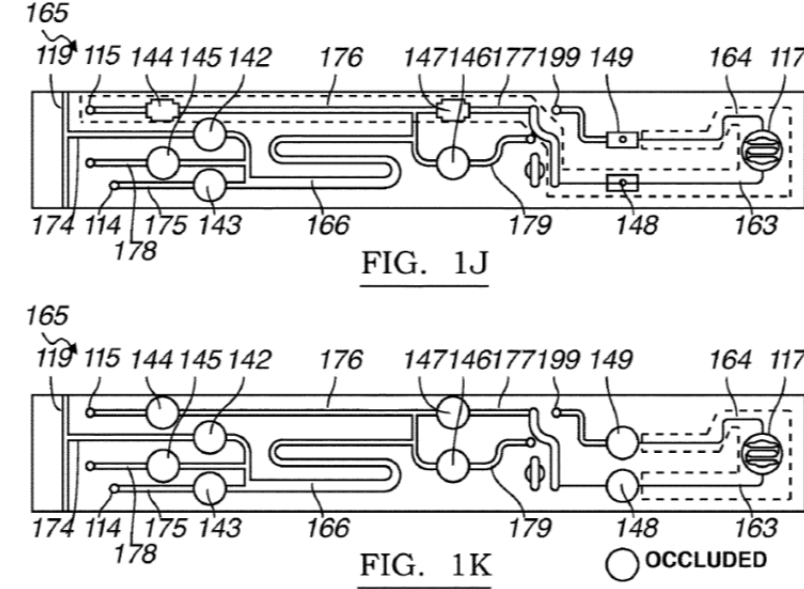
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> • Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads. • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic

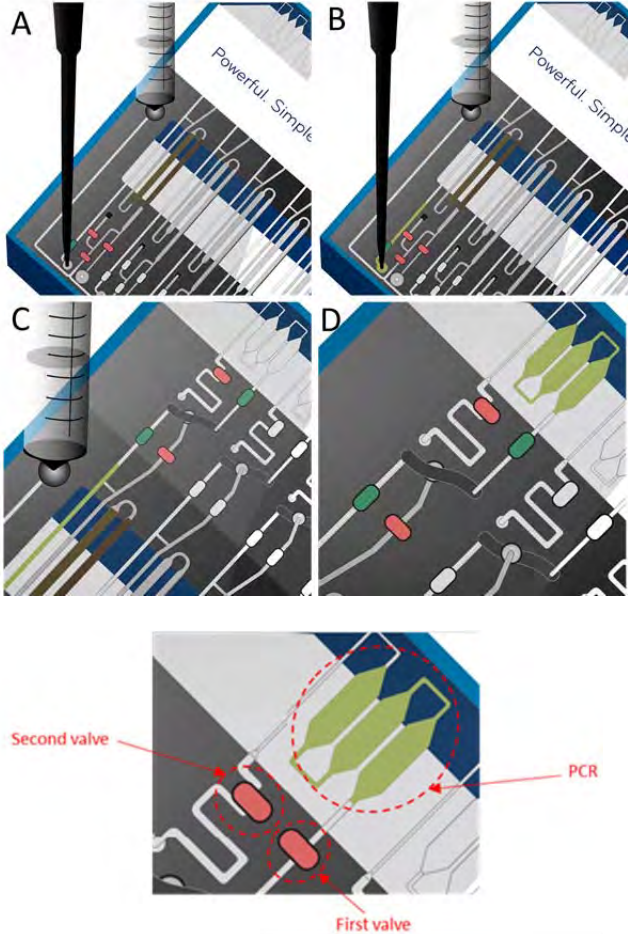
Claim	Claim Language	Infringement Evidence
		<p>pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,339,812 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”) • U.S. Patent No. 9,339,812 at 3:41-46 (“The detection chamber heaters 157, optical subsystem 180 and valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate analysis of the set of nucleic acid-reagent mixtures by a processor configured to display information on a user interface.”) • U.S. Patent No. 9,339,812 at 26:25-32 (“In a variation wherein the controller 272 is coupled to the molecular diagnostic module 130, the controller 272 preferably functions to automate reception of a microfluidic cartridge, heating of biological samples within the molecular diagnostic module 130 and the detection chambers 213, occlusion of fluidic pathways 220 by the valve actuation subsystem 170, and analysis of a set of nucleic acid-reagent mixtures by the optical subsystem 180.”) • U.S. Patent No. 9,339,812 at 33:3-39 (“Embodiments of the method 400 and

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		<p>variations thereof can be embodied and/or implemented at least in part by a machine configured to receive a computer-readable medium storing computer-readable instructions. The instructions are preferably executed by computer-executable components preferably integrated with the system 100 and one or more portions of the processor 273 and/or the controller 272. The computer-readable medium can be stored on any suitable computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices (CD or DVD), hard drives, floppy drives, or any suitable device. The computer-executable component is preferably a general or application specific processor, but any suitable dedicated hardware or hardware/firmware combination device can alternatively or additionally execute the instructions. The FIGURES illustrate the architecture, functionality and operation of possible implementations of systems, methods and computer program products according to preferred embodiments, example configurations, and variations thereof. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.”)</p> <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated

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		<p>surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 12:11-19 (“When not in operation, however, the normally closed position 43 is configured to prevent leakage and/or fluid

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		<p>bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) US Patent No. 9,738,887 at Figs. 1J and 1K:

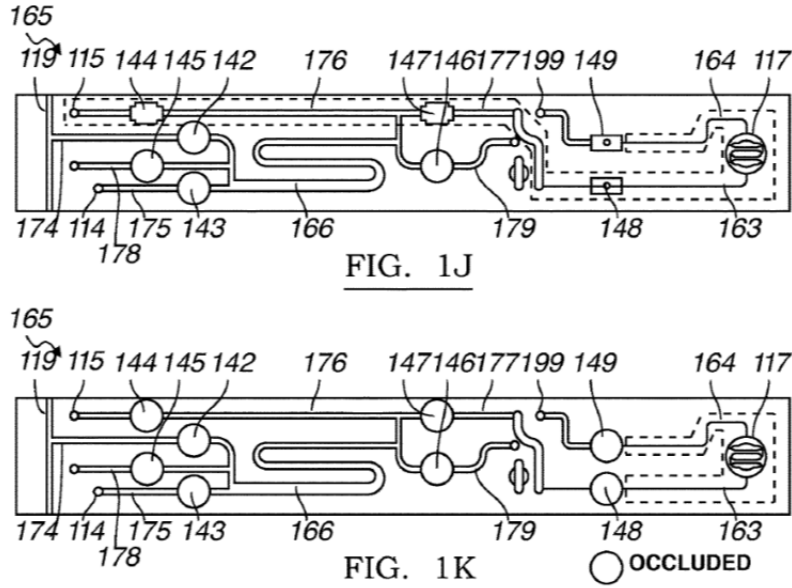
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1297 487 1428 527">FIG. 1J</p> <p data-bbox="1297 787 1428 828">FIG. 1K</p> <p data-bbox="1543 787 1711 820">○ OCCLUDED</p> <ul data-bbox="840 852 1890 1218" style="list-style-type: none"> • US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
1(l)	wherein the only ingress to and egress from the DNA manipulation zone is through the first and second valves, and	<p data-bbox="793 1258 1921 1323">In the accused system, the only ingress to and egress from the DNA manipulation zone is through the first and second valves.</p> <p data-bbox="793 1364 1858 1396"><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6,</p>

Claim	Claim Language	Infringement Evidence
		<p>2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p>The evidence consists of four panels (A, B, C, D) showing a microfluidic cartridge. Panels A and B show a pipette tip dispensing liquid into a channel. Panels C and D show the liquid moving through the channel. A larger diagram below shows the flow path with labels: 'Second valve', 'First valve', and 'PCR'.</p>

Claim	Claim Language	Infringement Evidence
		<p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> • Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads. • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber.

Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic

Claim	Claim Language	Infringement Evidence
		<p>pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
1(m)	wherein the computer-controlled heat source is in thermal contact with the DNA manipulation	In the accused system, the computer-controlled heat source is in thermal contact with the DNA manipulation zone.

Claim	Claim Language	Infringement Evidence
	zone; and	<p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6,</p>

Claim	Claim Language	Infringement Evidence
		<p>2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, last visited May 31, 2019, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> • “There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.” • “The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first

Claim	Claim Language	Infringement Evidence
		<p>substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics


Claim	Claim Language	Infringement Evidence
		<p>substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:21-32 (“As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection 30 chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.”) • U.S. Patent No. 9,499,896 at 9:11-19 (“As shown in FIGS. 1, 4A-4B, and 7A-7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heater-sensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the system 100, a set of elastic elements 160 configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.”) • U.S. Patent No. 9,499,896 at 12:20-31 (“In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply

Claim	Claim Language	Infringement Evidence
		<p>configured to supply voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistance-to-voltage conversion circuit because the UT750 PID controller requires voltage as an input for PID control.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 11:63-12:4 “As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100.”
1(n)	wherein the detector is configured to identify one or more polynucleotides within the DNA manipulation zone.	<p>The accused system comprises a detector configured to identify one or more polynucleotides within the DNA manipulation zone.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019 (Exhibit 14)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p>JFO_2018-10-25_8009-Rev-B_NeuMoDx-96-Spec-Sheet (Exhibit 21)</p>


Claim	Claim Language	Infringement Evidence																																				
		<table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>NeuMoDx_288_Spec_Sheet_R2.pdf (Exhibit 22)</p> <table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>US10041062 (Exhibit 33)</p> <ul style="list-style-type: none"> Claim 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising: a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode; a nozzle of a liquid handling subsystem; an optical subsystem; a cartridge heater; a magnet vertically aligned with the magnet receiving slot; and an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein: the nozzle of the liquid handling system is coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample, the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge, the optical subsystem interfaces with a second portion 	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
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		<p>of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and a third region of the cartridge is compressed between the cartridge heater and the cartridge platform.</p> <ul style="list-style-type: none"> • Claim 8. The system of claim 1, wherein the optical subsystem comprises at least one unit including an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the biological sample, and to transmit emitted light from the biological sample, through the emission filter, and toward the photodetector. <p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> • Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater. • Claim 11. The system of claim 1, wherein the molecular diagnostic module further comprises an optical subsystem comprising a first unit and a second unit, wherein each of the first unit and the second unit includes a set of excitation filters, a set of emission filters, a set of photodetectors aligned

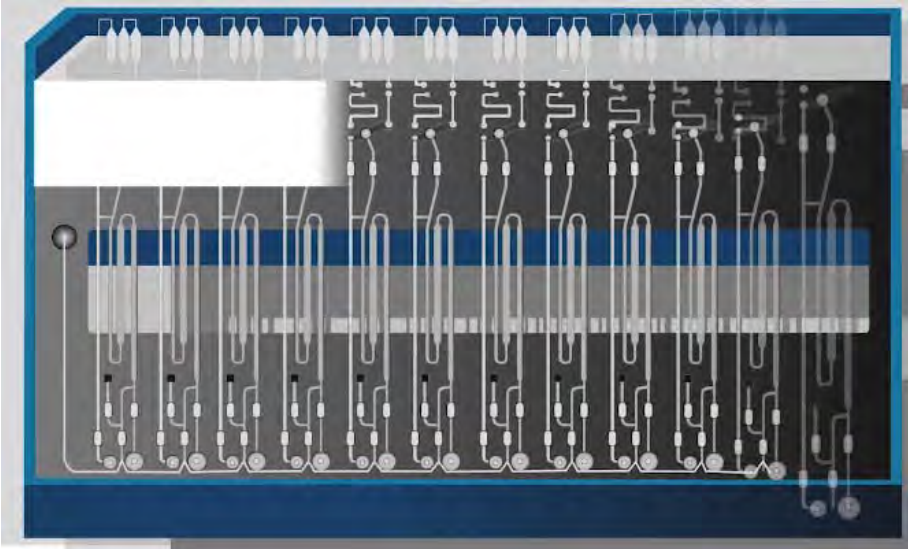
Claim	Claim Language	Infringement Evidence
		<p>with the set of emission filters, and a set of dichroic mirrors configured to reflect light from the set of excitation filters toward one of a set of nucleic acid-reagent mixtures at the cartridge, and to transmit emitted light from one of the set of nucleic acid-reagent mixtures, through at least one of the set of emission filters, and toward at least one of the set of photodetectors.</p> <ul style="list-style-type: none"> Claim 12. The system of claim 11, wherein the molecular diagnostic module further includes a set of detection chamber heaters configured to heat a set of detection chambers through the second surface of the cartridge, and wherein the optical subsystem is configured to receive light, emitted from the set of nucleic acid-reagent mixtures at the set of detection chambers, from the first surface of the cartridge.
18(a)	A device, comprising:	<p>To the extent the preamble is limiting, the accused instrument is a device.</p> <p><i>NeuMoDxTM Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p>

Claim	Claim Language	Infringement Evidence
		<div data-bbox="816 240 1845 1037">  </div> <p data-bbox="793 1076 1877 1149"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="846 1157 1877 1230" style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result.” <p data-bbox="793 1263 1906 1336"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul data-bbox="846 1344 1877 1411" style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result”

Claim	Claim Language	Infringement Evidence
		<p>platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.”</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “NeuMoDx™ Molecular Systems are versatile; in addition to IVD tests, our system can also be used as an open system to process Laboratory Developed Tests (LDTs) that have been created and validated by your lab.” <p><i>NeuMoDx™ Molecular Systems, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, last visited May 31, 2019, hyperlink at</i></p>

Claim	Claim Language	Infringement Evidence
		<p>https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> “There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.”
18(b)	a microfluidic process module;	<p>The accused device comprises a microfluidic process module</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic

Claim	Claim Language	Infringement Evidence
		<p>cartridge.”</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 24, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from ‘sample to result’. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>0600101_Rev-D-IFU-NeuMoDx-RELEASE-Solution-US-ONLY-FINAL-25Oct2018.pdf (Exhibit 20)</p> <ul style="list-style-type: none"> • “NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE... The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) and the dried Master Mix contained in a NeuMoDx test strip. The System then dispenses the prepared RT-PCR-ready mixture into the NeuMoDx

Claim	Claim Language	Infringement Evidence
		<p>Cartridge where Real-Time PCR occurs.”</p> <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59 

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		<p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater.
18(c)	a computer-controlled heat source; and	<p>The accused device comprises a computer-controlled heat source.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”.

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		<p>The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p>US9539576 (Exhibit 29)</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points. <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating


Claim	Claim Language	Infringement Evidence
		<p>layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:21-32 (“As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection 30 chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.”) • U.S. Patent No. 9,499,896 at 9:11-19 (“As shown in FIGS. 1, 4A-4B, and 7A-7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heater-sensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the system 100, a set of elastic elements 160

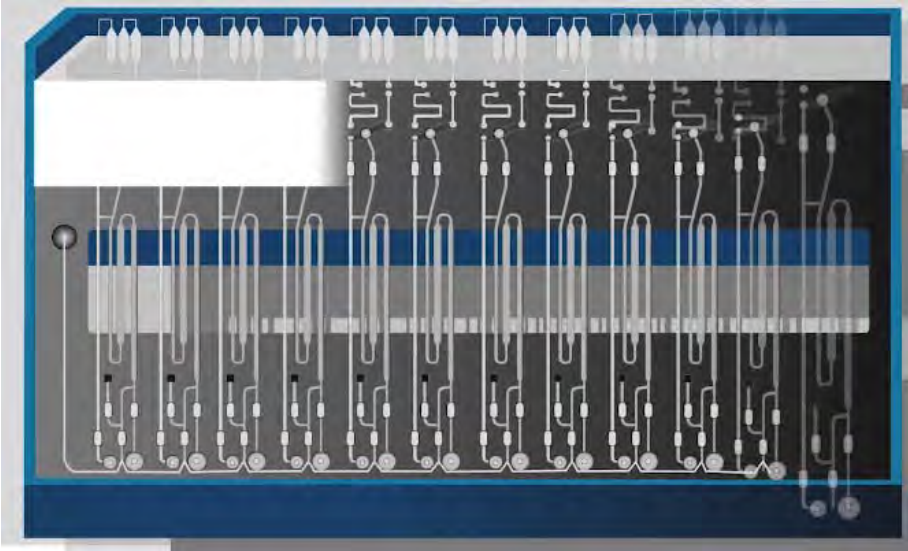
Claim	Claim Language	Infringement Evidence
		<p>configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 12:20-31 (“In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistance-to-voltage conversion circuit because the UT750 PID controller requires voltage as an input for PID control.”) U.S. Patent No. 9,499,896 at 11:63-12:4 “As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100.”
18(d)	a detector;	<p>The accused device comprises a detector.</p> <p><i>NeuMoDx™ Molecular Systems, NEUMODx, http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</i></p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems, NEUMODx, http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019 (Exhibit 14)</i></p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of

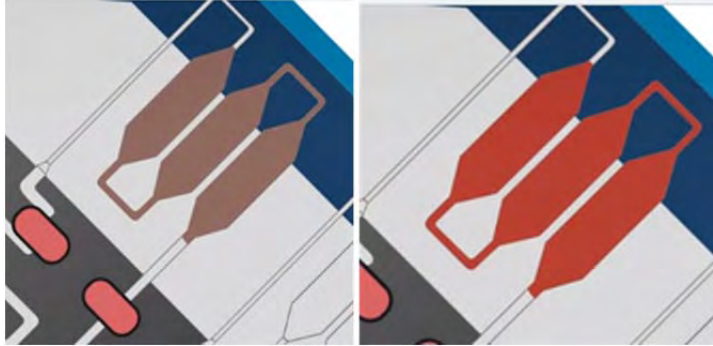
Claim	Claim Language	Infringement Evidence																																				
		<p>products of amplification.”</p> <p>JFO_2018-10-25_8009-Rev-B_NeuMoDx-96-Spec-Sheet (Exhibit 21)</p> <table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>NeuMoDx 288 Spec Sheet R2.pdf (Exhibit 22)</p> <table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>US10041062 (Exhibit 33)</p> <ul style="list-style-type: none"> Claim 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising: a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode; a nozzle of a liquid handling subsystem; an optical subsystem; a cartridge heater; a magnet vertically aligned with the magnet receiving slot; and an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein: the nozzle of the liquid handling system is 	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
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Claim	Claim Language	Infringement Evidence
		<p>coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample, the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge, the optical subsystem interfaces with a second portion of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and a third region of the cartridge is compressed between the cartridge heater and the cartridge platform.</p> <ul style="list-style-type: none"> Claim 8. The system of claim 1, wherein the optical subsystem comprises at least one unit including an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the biological sample, and to transmit emitted light from the biological sample, through the emission filter, and toward the photodetector. <p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater.

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> Claim 11. The system of claim 1, wherein the molecular diagnostic module further comprises an optical subsystem comprising a first unit and a second unit, wherein each of the first unit and the second unit includes a set of excitation filters, a set of emission filters, a set of photodetectors aligned with the set of emission filters, and a set of dichroic mirrors configured to reflect light from the set of excitation filters toward one of a set of nucleic acid-reagent mixtures at the cartridge, and to transmit emitted light from one of the set of nucleic acid-reagent mixtures, through at least one of the set of emission filters, and toward at least one of the set of photodetectors. Claim 12. The system of claim 11, wherein the molecular diagnostic module further includes a set of detection chamber heaters configured to heat a set of detection chambers through the second surface of the cartridge, and wherein the optical subsystem is configured to receive light, emitted from the set of nucleic acid-reagent mixtures at the set of detection chambers, from the first surface of the cartridge.
18(e)	wherein the microfluidic process module comprises: a zone configured to receive a sample and perform amplification of the sample;	<p>The accused device comprises a microfluidic process module comprising a zone configured to receive a sample and perform amplification of the sample.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a

Claim	Claim Language	Infringement Evidence
		<p data-bbox="890 235 1398 267">multi-sample microfluidic cartridge.”</p> <p data-bbox="793 342 1465 375"><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul data-bbox="844 383 1885 451" style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p data-bbox="802 1016 1675 1049"><i>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf</i> (Exhibit 19)</p> <ul data-bbox="844 1057 1923 1344" style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p data-bbox="793 1385 1860 1417"><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6,</p>

Claim	Claim Language	Infringement Evidence
		<p>2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

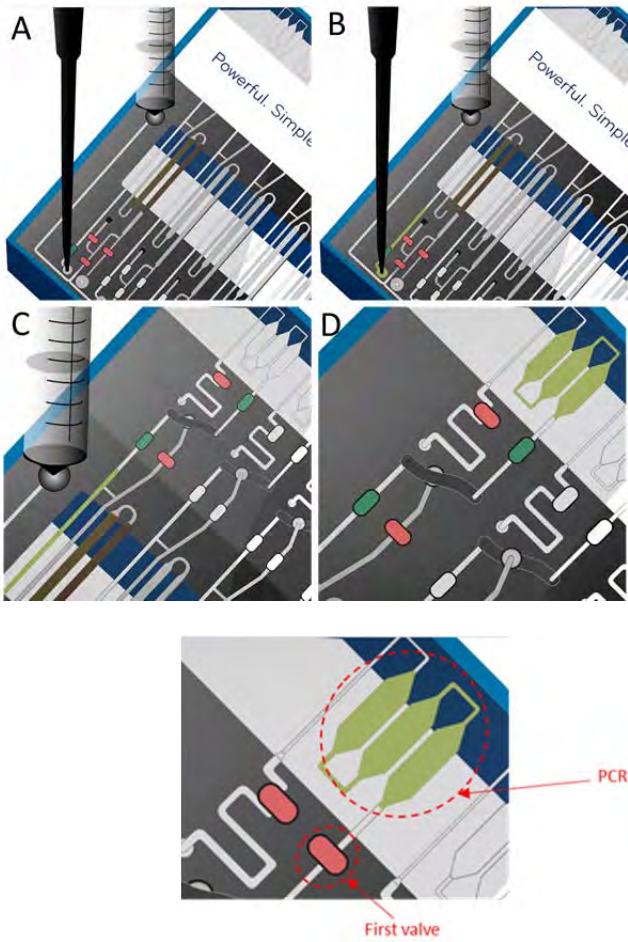
Claim	Claim Language	Infringement Evidence
		 <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> • Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. • Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the

Claim	Claim Language	Infringement Evidence
		<p>second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <ul style="list-style-type: none"> Claim 11. The cartridge of claim 10, further comprising 1) a heating region defined as a recessed region of the first layer that is parallel to the set of voids of the corrugated surface, and 2) a vent region, such that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber. <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the

Claim	Claim Language	Infringement Evidence
		<p>waste chamber, and to be occluded upon deformation of the elastomeric layer.</p> <ul style="list-style-type: none"> • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the

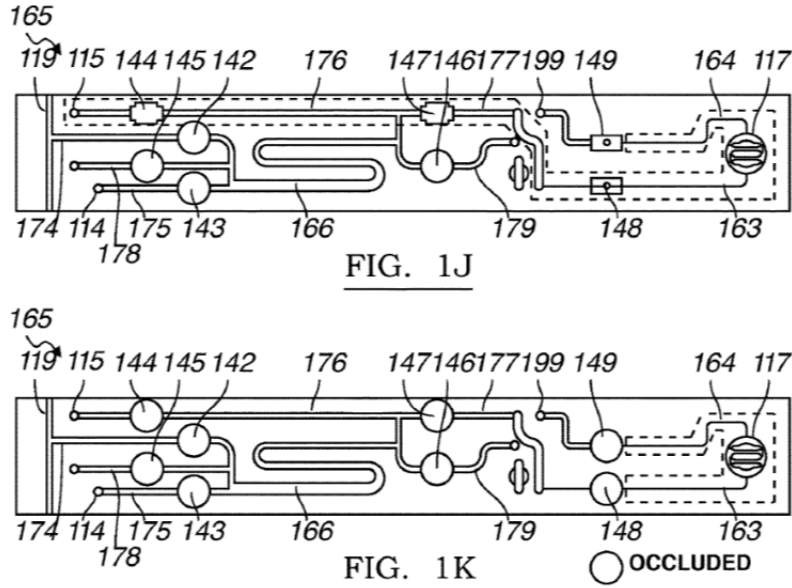
Claim	Claim Language	Infringement Evidence
		<p>detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”) U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) U.S. Patent No. 9,050,594 at 10:49-65 (“The cartridge heater 153 functions to transfer heat to a heating region 224 of a microfluidic cartridge 210, for inducing a pH shift to release bound nucleic acids from magnetic beads within the heating region 224... The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”)

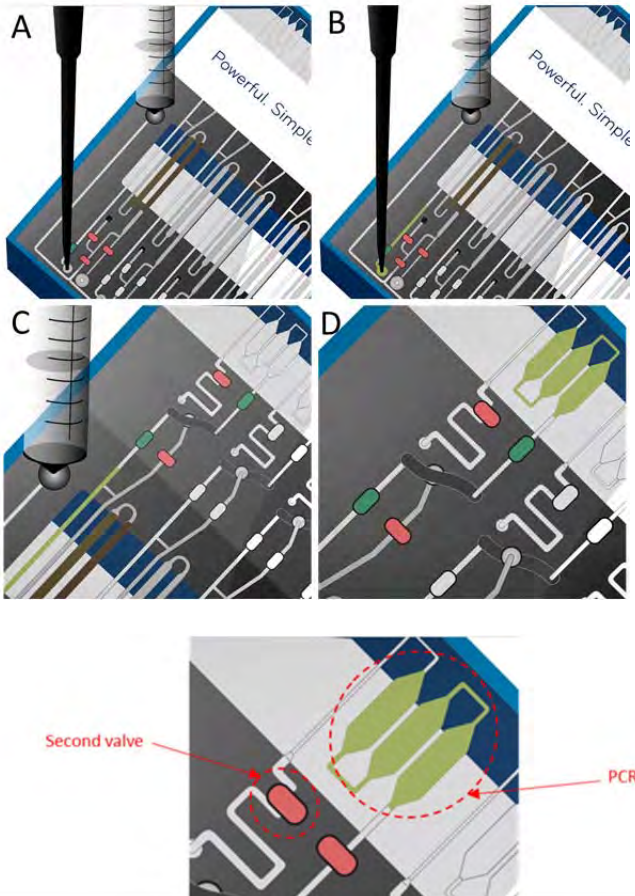
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) U.S. Patent No. 9,050,594 at 29:44-47 (“In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.”)
18(f)	[the microfluidic process module comprises] a first valve upstream of the zone;	<p>The accused device comprises a microfluidic process module comprising a first valve upstream of the zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

Claim	Claim Language	Infringement Evidence
		 <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve

Claim	Claim Language	Infringement Evidence
		<p>guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as

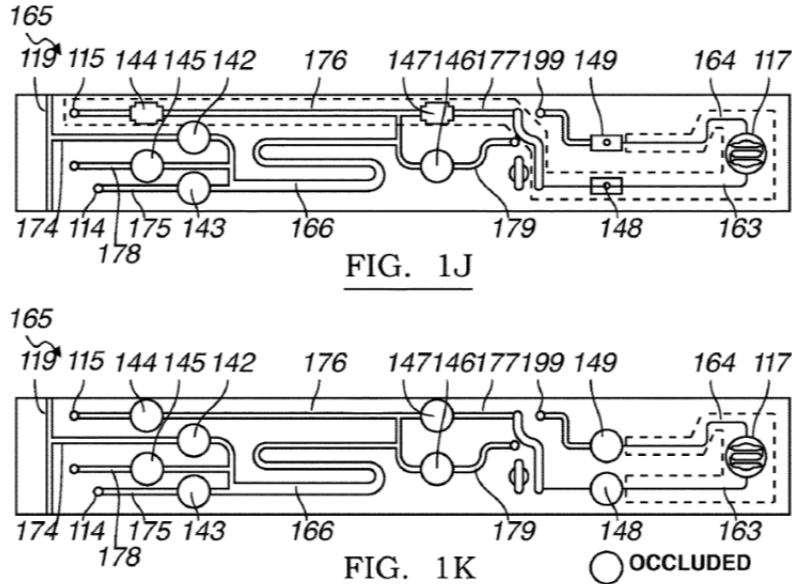
Claim	Claim Language	Infringement Evidence
		<p>shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

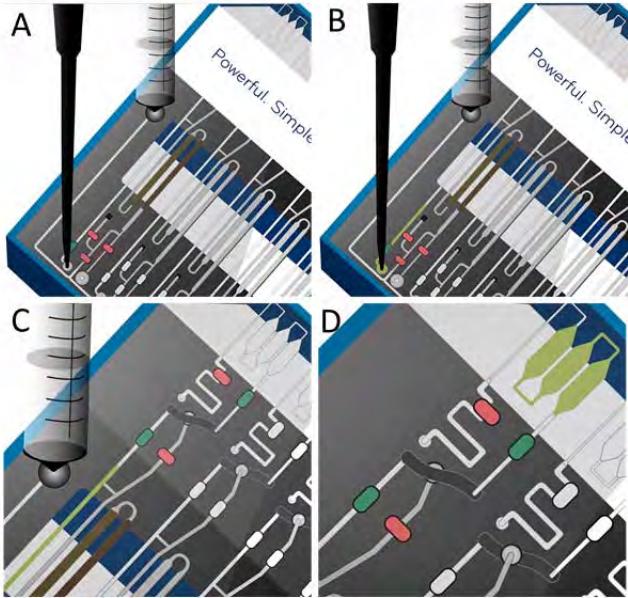
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1297 488 1430 521">FIG. 1J</p> <p data-bbox="1297 789 1430 821">FIG. 1K</p> <p data-bbox="1541 789 1709 821">○ OCCLUDED</p> <ul data-bbox="842 854 1919 1219" style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
18(g)	[the microfluidic process module comprises] a second valve downstream of the zone; and	The accused device comprises a microfluidic process module comprising a second valve downstream of the zone.

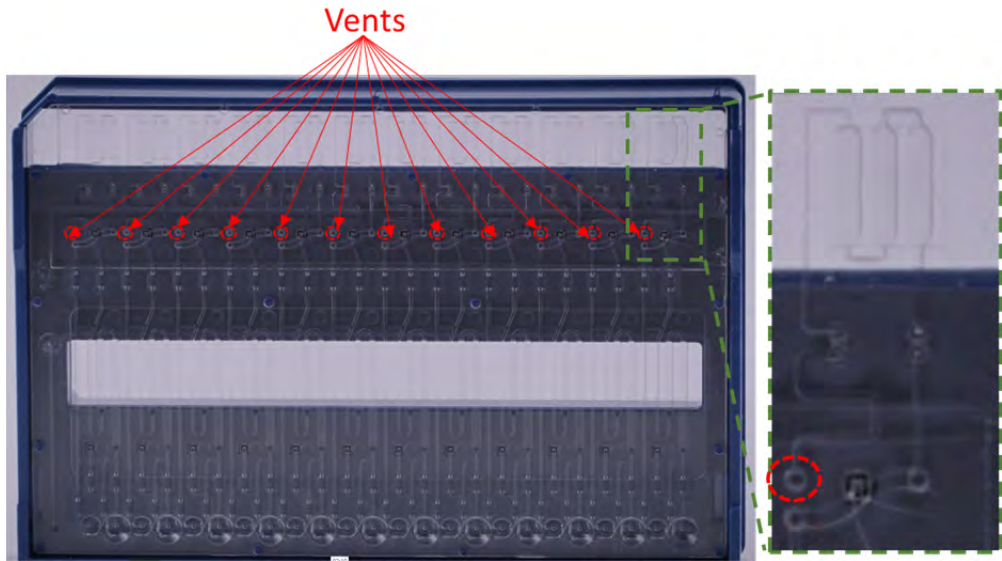
Claim	Claim Language	Infringement Evidence
		<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p>The evidence consists of four panels (A, B, C, D) showing a microfluidic cartridge. Panel A shows a pipette tip approaching the cartridge. Panel B shows the pipette tip dispensing a droplet. Panel C shows the droplet moving through a channel. Panel D shows the droplet moving through a channel with a valve. A larger inset below shows a close-up of a valve and a PCR chamber, with labels 'Second valve' and 'PCR'.</p>

Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second

Claim	Claim Language	Infringement Evidence
		<p>truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

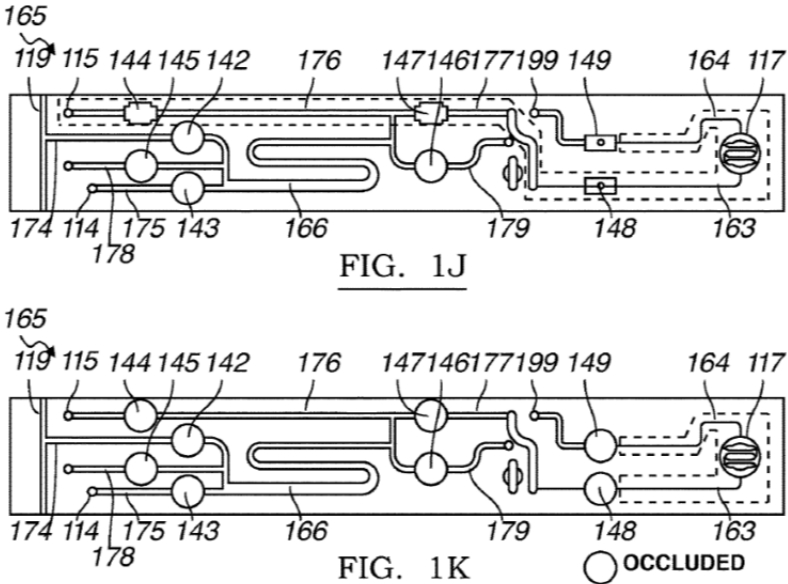
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1297 488 1430 521">FIG. 1J</p> <p data-bbox="1297 789 1430 821">FIG. 1K</p> <p data-bbox="1541 789 1703 821">○ OCCLUDED</p> <ul data-bbox="842 854 1923 1211" style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
18(h)	[the microfluidic process module comprises] a vent separated from the first valve by the second valve;	<p data-bbox="793 1260 1814 1325">The accused device comprises a microfluidic process module comprising a vent separated from the first valve by the second valve.</p> <p data-bbox="793 1365 1860 1398"><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6,</p>

Claim	Claim Language	Infringement Evidence
		<p>2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>  <p>On information and belief, the accused cartridge comprises a vent disposed within the DNA manipulation module and separated from the upstream channel by the first and second valves.</p> <ul style="list-style-type: none"> • <i>Id.</i> at 2:10

Claim	Claim Language	Infringement Evidence
		 <p>The image shows a cartridge assembly with a series of vents along the top edge. Red arrows point from the word 'Vents' to these vents. A dashed green box highlights a specific component on the right side of the assembly, which is a circular feature with a red dashed circle around it.</p> <p>US9101930 (Exhibit 25)</p> <ul style="list-style-type: none"> Claim 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising: a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer; a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, are each at least partially separated from the corrugated surface of the intermediate substrate by an elastomeric layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate. Claim 11. The cartridge of claim 10, wherein the first layer is a unitary

Claim	Claim Language	Infringement Evidence
		<p>construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <ul style="list-style-type: none"> • Claim 13. The cartridge of claim 11, further comprising a heating region as a recessed region of the first layer that is parallel to the set of parallel voids of the corrugated surface, and a vent region, such that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber. • Claim 15. The cartridge of claim 13, wherein at least of the first fluidic pathway and the second fluidic pathway is coupled to an end vent configured to provide fine metering of fluid flow. <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent

Claim	Claim Language	Infringement Evidence
		<p>port, the fluid port, and the detection chamber.</p> <ul style="list-style-type: none"> • Claim 10. The cartridge of claim 1, wherein a terminal portion of the fluidic pathway is coupled to an end vent, configured to provide fine metering of fluid flow. • U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) • U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1249 488 1381 521">FIG. 1J</p> <p data-bbox="1249 789 1381 821">FIG. 1K</p> <p data-bbox="1493 789 1661 821">○ OCCLUDED</p> <ul data-bbox="842 854 1892 951" style="list-style-type: none"> • U.S. Patent No. 8,738,887 at 15:4-6 (“A fluidic pathway 165 may also further comprise an end vent 199, which functions to prevent any fluid from escaping the microfluidic channel.”)
18(i)	a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the zone when amplification of the sample occurs in the zone,	<p data-bbox="793 967 1921 1065">The accused device comprises a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the zone when amplification of the sample occurs in the zone</p> <p data-bbox="793 1105 1921 1179"><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul data-bbox="842 1187 1892 1401" style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that

Claim	Claim Language	Infringement Evidence
		<p>fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

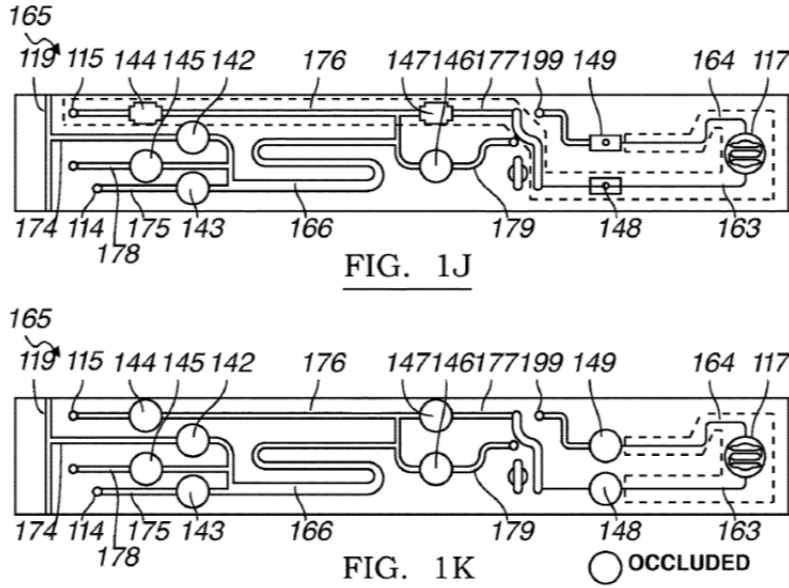
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> • Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads. • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic

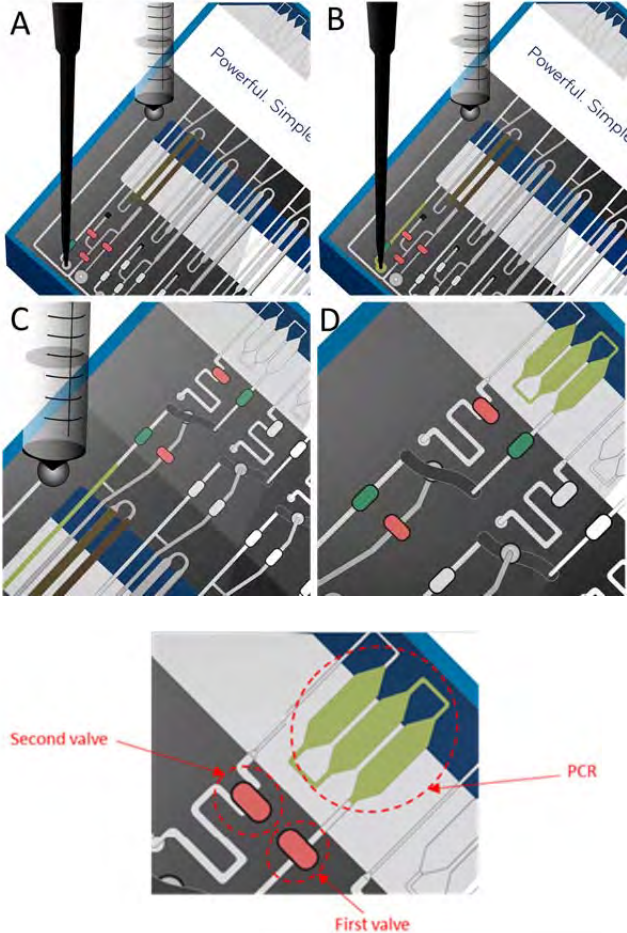
Claim	Claim Language	Infringement Evidence
		<p>pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,339,812 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”) U.S. Patent No. 9,339,812 at 3:41-46 (“The detection chamber heaters 157, optical subsystem 180 and valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate analysis of the set of nucleic acid-reagent mixtures by a processor configured to display information on a user interface.”) U.S. Patent No. 9,339,812 at 26:25-32 (“In a variation wherein the controller 272 is coupled to the molecular diagnostic module 130, the controller 272 preferably functions to automate reception of a microfluidic cartridge, heating of biological samples within the molecular diagnostic module 130 and the detection chambers 213, occlusion of fluidic pathways 220 by the valve actuation subsystem 170, and analysis of a set of nucleic acid-reagent mixtures by the optical subsystem 180.”) U.S. Patent No. 9,339,812 at 33:3-39 (“Embodiments of the method 400 and

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		<p>variations thereof can be embodied and/or implemented at least in part by a machine configured to receive a computer-readable medium storing computer-readable instructions. The instructions are preferably executed by computer-executable components preferably integrated with the system 100 and one or more portions of the processor 273 and/or the controller 272. The computer-readable medium can be stored on any suitable computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices (CD or DVD), hard drives, floppy drives, or any suitable device. The computer-executable component is preferably a general or application specific processor, but any suitable dedicated hardware or hardware/firmware combination device can alternatively or additionally execute the instructions. The FIGURES illustrate the architecture, functionality and operation of possible implementations of systems, methods and computer program products according to preferred embodiments, example configurations, and variations thereof. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.”)</p> <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated

Claim	Claim Language	Infringement Evidence
		<p>surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 12:11-19 (“When not in operation, however, the normally closed position 43 is configured to prevent leakage and/or fluid

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		<p>bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) US Patent No. 9,738,887 at Figs. 1J and 1K:

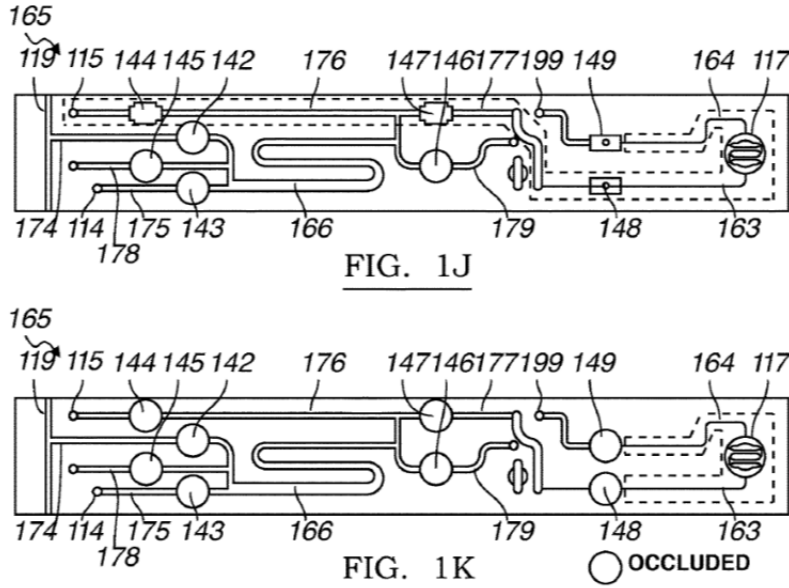
Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
18(j)	wherein the only ingress to and egress from the zone is through the first and second valves;	<p>In the accused device, the only ingress to and egress from the zone is through the first and second valves</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6,</p>

Claim	Claim Language	Infringement Evidence
		<p>2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 

Claim	Claim Language	Infringement Evidence
		<p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> • Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads. • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber.

Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic

Claim	Claim Language	Infringement Evidence
		<p>pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
18(k)	wherein the computer-controlled heat source is in thermal contact with the zone; and	<p>In the accused device, the computer-controlled heat source is in thermal contact with the zone.</p> <p><i>NeuMoDx™ Molecular Systems, NEUMODX, http://www.neumodx.com/our-solutions/,</i></p>

Claim	Claim Language	Infringement Evidence
		<p>last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO </p>

Claim	Claim Language	Infringement Evidence
		<p>NeuMoDxTM WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p><i>NeuMoDxTM Molecular Systems</i>, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, last visited May 31, 2019, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> • “There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.” • “The NeuMoDxTM 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDxTM 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate

Claim	Claim Language	Infringement Evidence
		<p>connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the


Claim	Claim Language	Infringement Evidence
		<p>set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:21-32 (“As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection 30 chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.”) • U.S. Patent No. 9,499,896 at 9:11-19 (“As shown in FIGS. 1, 4A-4B, and 7A-7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heater-sensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the system 100, a set of elastic elements 160 configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.”) • U.S. Patent No. 9,499,896 at 12:20-31 (“In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply voltage to the resistance-to-voltage conversion circuit.

Claim	Claim Language	Infringement Evidence
		<p>In the specific example, the controller 165 comprises a resistance-to-voltage conversion circuit because the UT750 PID controller requires voltage as an input for PID control.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 11:63-12:4 “As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100.”
18(l)	wherein the detector is configured to identify one or more polynucleotides within the zone.	<p>In the accused device, the detector is configured to identify one or more polynucleotides within the zone.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019 (Exhibit 14)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p>JFO_2018-10-25_8009-Rev-B_NeuMoDx-96-Spec-Sheet (Exhibit 21)</p>


Claim	Claim Language	Infringement Evidence																																				
		<table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>NeuMoDx_288_Spec_Sheet_R2.pdf (Exhibit 22)</p> <table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>US10041062 (Exhibit 33)</p> <ul style="list-style-type: none"> Claim 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising: a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode; a nozzle of a liquid handling subsystem; an optical subsystem; a cartridge heater; a magnet vertically aligned with the magnet receiving slot; and an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein: the nozzle of the liquid handling system is coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample, the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge, the optical subsystem interfaces with a second portion 	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
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Claim	Claim Language	Infringement Evidence
		<p>of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and a third region of the cartridge is compressed between the cartridge heater and the cartridge platform.</p> <ul style="list-style-type: none"> • Claim 8. The system of claim 1, wherein the optical subsystem comprises at least one unit including an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the biological sample, and to transmit emitted light from the biological sample, through the emission filter, and toward the photodetector. <p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> • Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater. • Claim 11. The system of claim 1, wherein the molecular diagnostic module further comprises an optical subsystem comprising a first unit and a second unit, wherein each of the first unit and the second unit includes a set of excitation filters, a set of emission filters, a set of photodetectors aligned

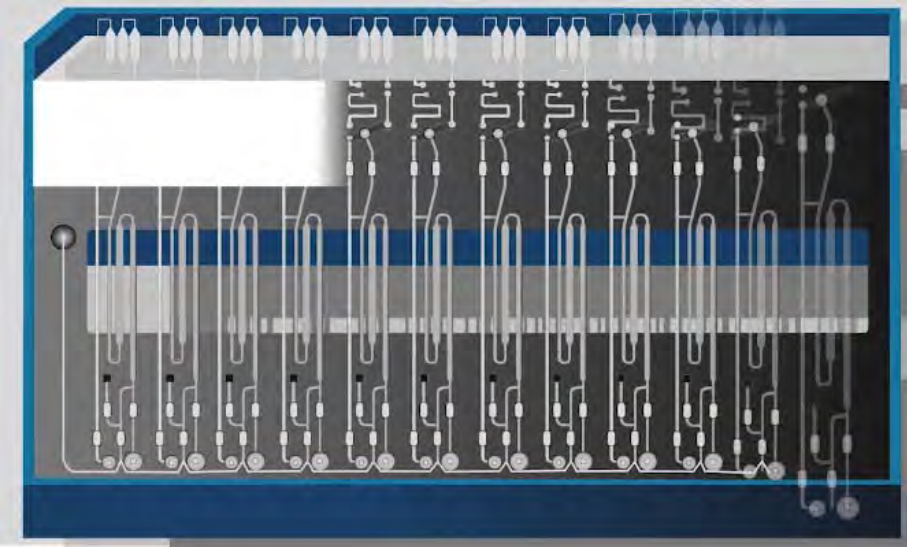
Claim	Claim Language	Infringement Evidence
		<p>with the set of emission filters, and a set of dichroic mirrors configured to reflect light from the set of excitation filters toward one of a set of nucleic acid-reagent mixtures at the cartridge, and to transmit emitted light from one of the set of nucleic acid-reagent mixtures, through at least one of the set of emission filters, and toward at least one of the set of photodetectors.</p> <ul style="list-style-type: none"> Claim 12. The system of claim 11, wherein the molecular diagnostic module further includes a set of detection chamber heaters configured to heat a set of detection chambers through the second surface of the cartridge, and wherein the optical subsystem is configured to receive light, emitted from the set of nucleic acid-reagent mixtures at the set of detection chambers, from the first surface of the cartridge.
19(a)	A system, comprising:	<p>To the extent the preamble is limiting, the accused instruments are a system.</p> <p><i>NeuMoDxTM Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p>

Claim	Claim Language	Infringement Evidence
		<div data-bbox="816 240 1845 1040">  </div> <p data-bbox="789 1076 1879 1149"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="842 1157 1890 1230" style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result.” <p data-bbox="789 1263 1906 1336"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul data-bbox="842 1344 1879 1411" style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result”

Claim	Claim Language	Infringement Evidence
		<p>platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.”</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “NeuMoDx™ Molecular Systems are versatile; in addition to IVD tests, our system can also be used as an open system to process Laboratory Developed Tests (LDTs) that have been created and validated by your lab.” <p><i>NeuMoDx™ Molecular Systems, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, last visited May 31, 2019, hyperlink at</i></p>

Claim	Claim Language	Infringement Evidence
		<p>https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> “There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.”
19(b)	a microfluidic device;	<p>The accused system comprises a microfluidic device.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic

Claim	Claim Language	Infringement Evidence
		<p>cartridge.”</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 24, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from ‘sample to result’. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>0600101_Rev-D-IFU-NeuMoDx-RELEASE-Solution-US-ONLY-FINAL-25Oct2018.pdf (Exhibit 20)</p> <ul style="list-style-type: none"> • “NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE... The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) and the dried Master Mix contained in a NeuMoDx test strip. The System then dispenses the prepared RT-PCR-ready mixture into the NeuMoDx

Claim	Claim Language	Infringement Evidence
		<p>Cartridge where Real-Time PCR occurs.”</p> <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59 

Claim	Claim Language	Infringement Evidence
		<p>US10041062 (Exhibit 33)</p> <ul style="list-style-type: none"> Claim 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising: a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode; a nozzle of a liquid handling subsystem; an optical subsystem; a cartridge heater; a magnet vertically aligned with the magnet receiving slot; and an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein: the nozzle of the liquid handling system is coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample, the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge, the optical subsystem interfaces with a second portion of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and a third region of the cartridge is compressed between the cartridge heater and the cartridge platform. <p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a

Claim	Claim Language	Infringement Evidence
		<p>magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater.</p>
19(c)	a computer-controlled heat source; and	<p>The accused system comprises a computer-controlled heat source.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and

Claim	Claim Language	Infringement Evidence
		<p>consumables.”</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the

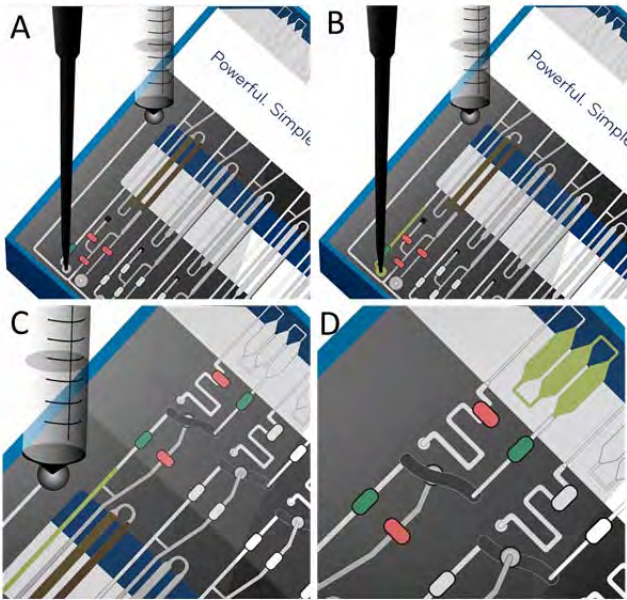
Claim	Claim Language	Infringement Evidence
		<p>second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first

Claim	Claim Language	Infringement Evidence
		<p>substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 2:21-32 (“As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection 30 chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.”) U.S. Patent No. 9,499,896 at 9:11-19 (“As shown in FIGS. 1, 4A-4B, and 7A-7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heater-sensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the system 100, a set of elastic elements 160 configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.”) U.S. Patent No. 9,499,896 at 12:20-31 (“In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistance-to-voltage conversion circuit because the UT750 PID controller requires voltage as an

Claim	Claim Language	Infringement Evidence																		
		<p>input for PID control.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 11:63-12:4 “As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100.” 																		
19(d)	a detector;	<p>The accused system comprises a detector</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019 (Exhibit 14)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p>JFO_2018-10-25_8009-Rev-B NeuMoDx-96-Spec-Sheet (Exhibit 21)</p> <table border="1"> <thead> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> </thead> <tbody> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </tbody> </table>	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
Optical Wavelengths	Excitation (nm)	Emission (nm)																		
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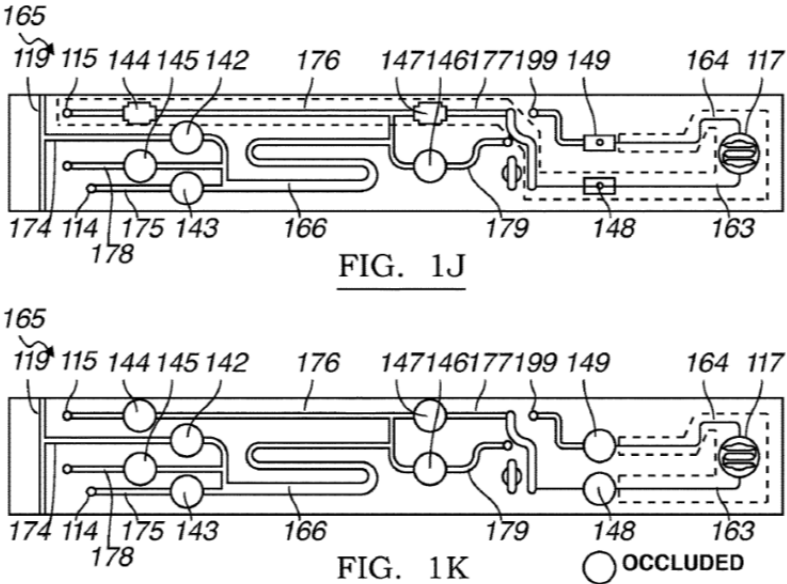
Claim	Claim Language	Infringement Evidence																		
		<p>NeuMoDx_288_Spec_Sheet_R2.pdf (Exhibit 22)</p> <table border="1"> <thead> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> </thead> <tbody> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </tbody> </table> <p>US10041062 (Exhibit 33)</p> <ul style="list-style-type: none"> Claim 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising: a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode; a nozzle of a liquid handling subsystem; an optical subsystem; a cartridge heater; a magnet vertically aligned with the magnet receiving slot; and an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein: the nozzle of the liquid handling system is coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample, the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge, the optical subsystem interfaces with a second portion of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and a third region of the cartridge is compressed between the cartridge heater and the cartridge platform. Claim 8. The system of claim 1, wherein the optical subsystem comprises at least one unit including an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the biological sample, and to transmit emitted light from the biological sample, through 	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
Optical Wavelengths	Excitation (nm)	Emission (nm)																		
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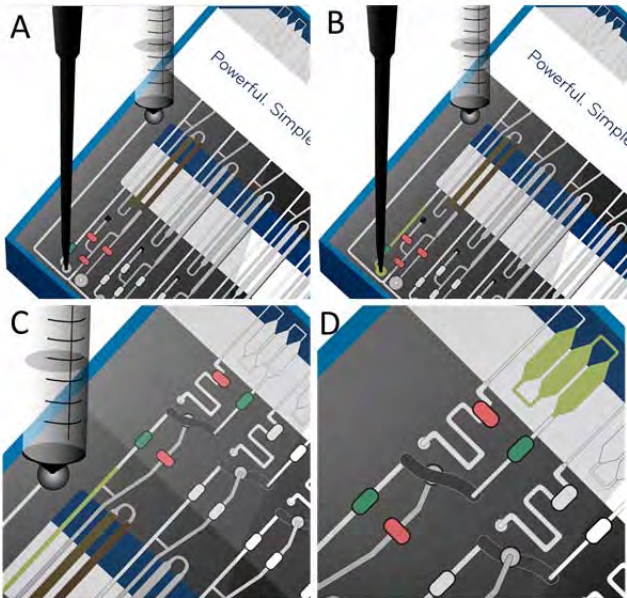
Claim	Claim Language	Infringement Evidence
		<p>the emission filter, and toward the photodetector.</p> <p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> • Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater. • Claim 11. The system of claim 1, wherein the molecular diagnostic module further comprises an optical subsystem comprising a first unit and a second unit, wherein each of the first unit and the second unit includes a set of excitation filters, a set of emission filters, a set of photodetectors aligned with the set of emission filters, and a set of dichroic mirrors configured to reflect light from the set of excitation filters toward one of a set of nucleic acid-reagent mixtures at the cartridge, and to transmit emitted light from one of the set of nucleic acid-reagent mixtures, through at least one of the set of emission filters, and toward at least one of the set of photodetectors. • Claim 12. The system of claim 11, wherein the molecular diagnostic module further includes a set of detection chamber heaters configured to heat a set of detection chambers through the second surface of the cartridge, and wherein the

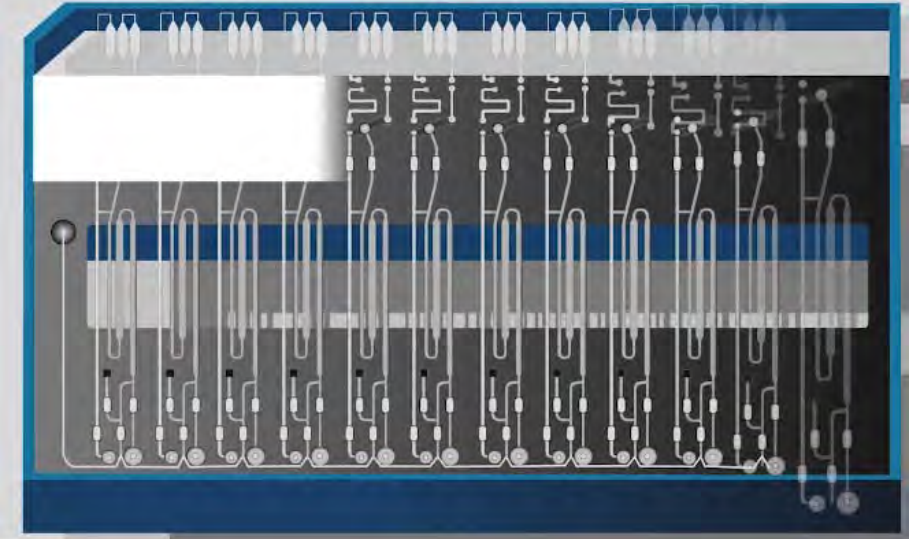
Claim	Claim Language	Infringement Evidence
		<p>optical subsystem is configured to receive light, emitted from the set of nucleic acid-reagent mixtures at the set of detection chambers, from the first surface of the cartridge.</p>
19(e)	wherein the microfluidic device comprises: an upstream channel;	<p>The accused system comprises a microfluidic device comprising an upstream channel</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at Abstract (“A microfluidic cartridge, configured to

Claim	Claim Language	Infringement Evidence
		<p>facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • U.S. Patent No. 9,738,887 at 15:31-35 (“The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.”) • U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165

Claim	Claim Language	Infringement Evidence
		<p>may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

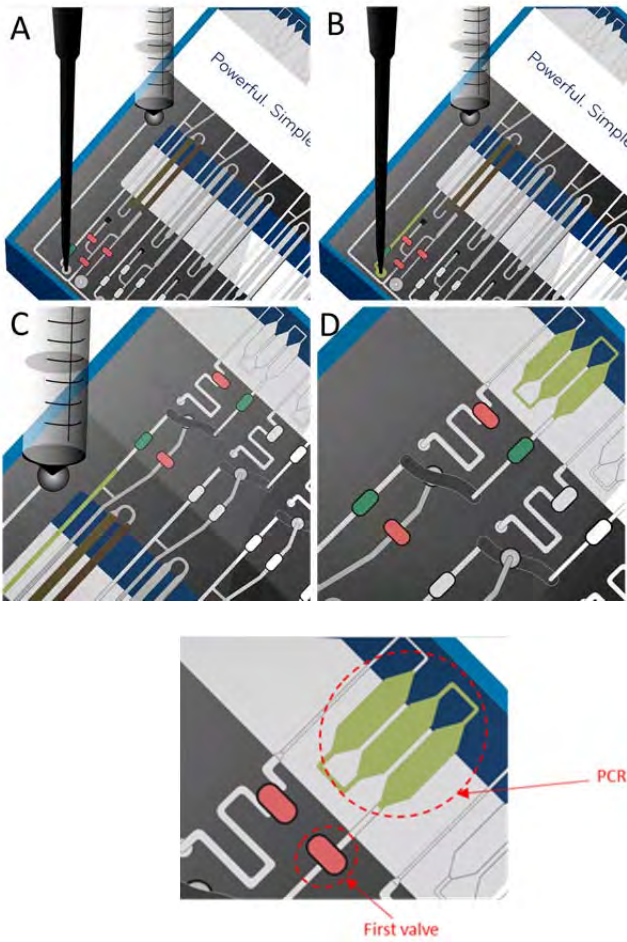
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1249 487 1375 527">FIG. 1J</p> <p data-bbox="1249 787 1375 828">FIG. 1K</p> <p data-bbox="1491 787 1659 820">○ OCCLUDED</p> <ul data-bbox="840 852 1890 1031" style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 23:36-41 (“Each detection chamber 117 of the specific embodiment is identical and comprised of three interconnected channels, configured in a circular arrangement, with each of the interconnected channels approximately 0.4 mm deep and 1.6 mm wide at its widest point, resulting in a total volume of ~10 nL for each detection chamber 117.”)
19(f)	[the microfluidic device comprises] a DNA manipulation zone located downstream from the upstream channel and configured to perform PCR amplification of a sample;	<p data-bbox="793 1112 1919 1218">The accused system comprises a microfluidic device comprising a DNA manipulation zone located downstream from the upstream channel and configured to perform PCR amplification of a sample</p> <p data-bbox="793 1258 1919 1396"><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

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		<ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.”

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		 <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 • U.S. Patent No. 9,738,887 at Abstract (“A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region.”)

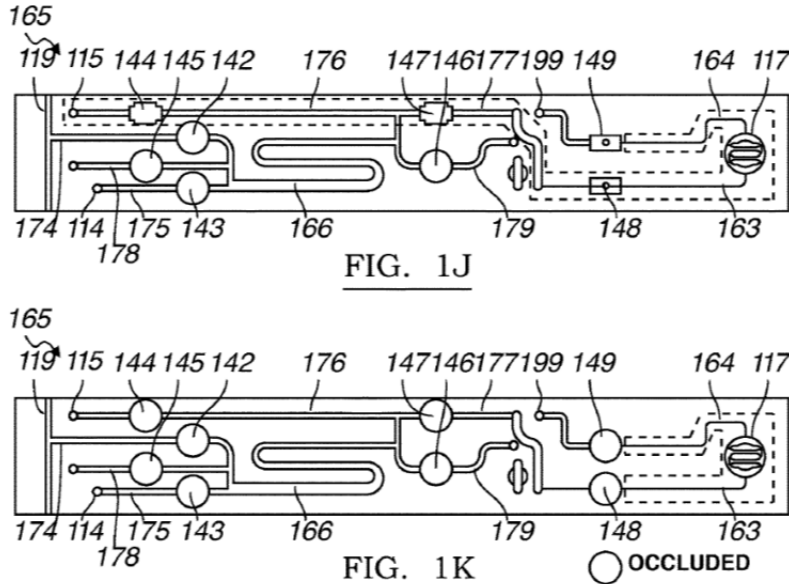
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 2:36-3:5. (“As shown in FIGS. 1A-1C, an embodiment of a microfluidic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample port-reagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; and a set of fluidic pathways 160, each formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140.. In a specific application, the microfluidic cartridge 100 can be used to facilitate a PCR procedure for analysis of a sample containing nucleic acids.”) • U.S. Patent No. 9,738,887 at 13:7-18. (“The top layer 110 of an embodiment of the microfluidic cartridge 100 functions to accommodate elements involved in performing a molecular diagnostic procedure (e.g. PCR), such that a sample containing nucleic acids, passing through the cartridge, can be manipulated by the elements involved in performing the molecular diagnostic procedure. The top layer 110 is preferably composed of a structurally rigid/stiff material with low autofluorescence, such that the top layer 110 does not interfere with sample detection by fluorescence or chemiluminescence techniques, and an appropriate glass transition temperature and chemical compatibility for PCR or other amplification techniques.”) • U.S. Patent No. 9,738,887 at 13:35-42. (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • U.S. Patent No. 9,738,887 at 15:29-39 (“The segments may be arranged in at least one of several configurations to facilitate isolation, processing, and

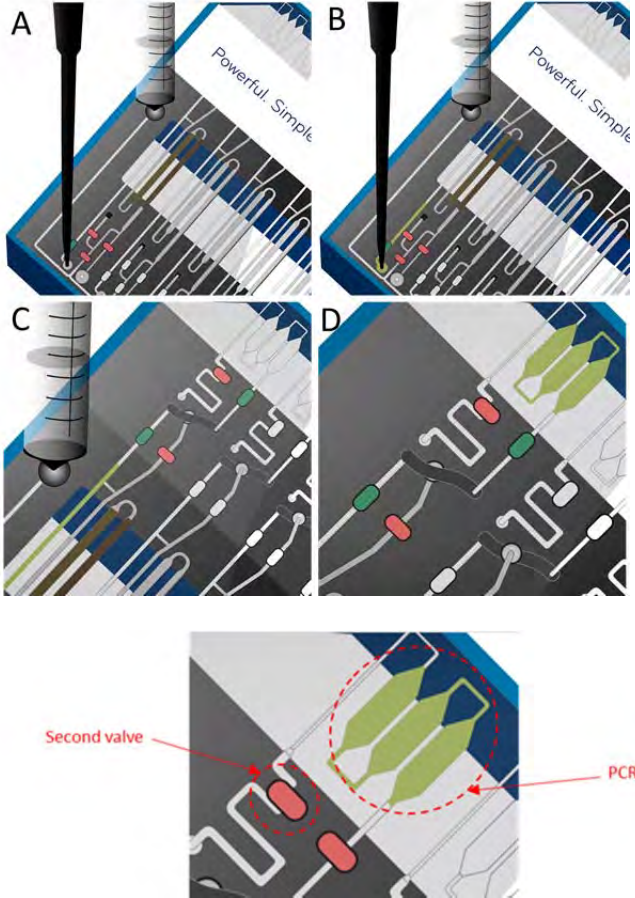
Claim	Claim Language	Infringement Evidence
		<p>amplification of a nucleic acid sample ...”).</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 23:20-24 (“The top layer 110 of the specific embodiment of the microfluidic cartridge 100 functions preferably as described in Section 1.1, and is composed of polypropylene with low autofluorescence and a glass transition temperature suitable for PCR.”) • U.S. Patent No. 9,738,887 at 23:36-41 (“Each detection chamber 117 of the specific embodiment is identical and comprised of three interconnected channels, configured in a circular arrangement, with each of the interconnected channels approximately 0.4 mm deep and 1.6 mm wide at its widest point, resulting in a total volume of -10 mL for each detection chamber 117.”) • U.S. Patent No. 9,738,887 at 24:1-11 (“In the specific embodiment, the intermediate substrate 120 is composed of a polypropylene material to minimize cost and simplify assembly, and in the orientation shown in FIG. 11B, the top of the intermediate substrate 120 is 1.5 mm thick. The film layer 125, partially separating the intermediate substrate 120 from the top layer 110 is a polypropylene film with a nominal thickness of 50 microns. The film layer 125 is able to withstand temperatures of up to 95° C. encountered during fabrication and during an intended PCR procedure, while being thermally bondable to the top layer 110.”)
19(g)	[the microfluidic device comprises] a first valve disposed upstream of the DNA manipulation zone; and	<p>The accused system comprises a microfluidic device comprising a first valve disposed upstream of the DNA manipulation zone</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

Claim	Claim Language	Infringement Evidence
		 <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve

Claim	Claim Language	Infringement Evidence
		<p>guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as

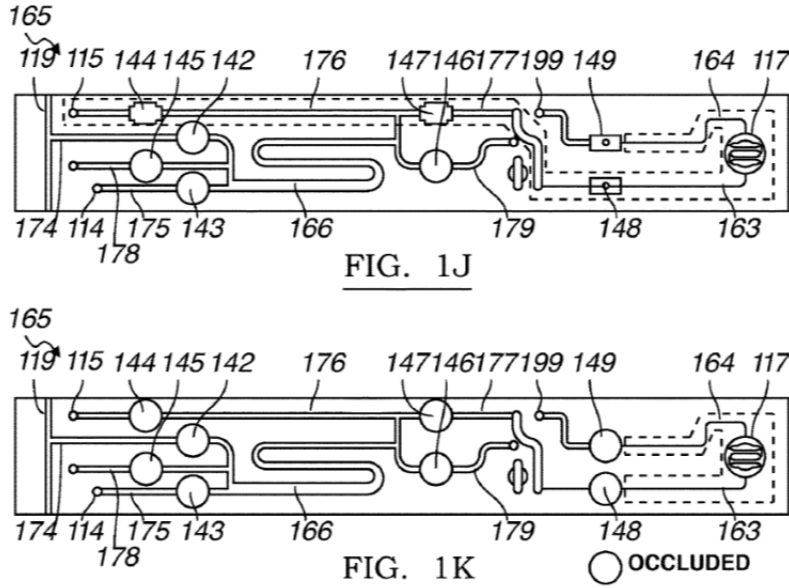
Claim	Claim Language	Infringement Evidence
		<p>shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)at Figs. 1J and 1K:</p>

Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1297 488 1430 521">FIG. 1J</p> <p data-bbox="1297 789 1430 821">FIG. 1K</p> <p data-bbox="1541 789 1703 821">○ OCCLUDED</p> <ul style="list-style-type: none"> <li data-bbox="842 854 1919 1211">U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
19(h)	[the microfluidic device comprises] a second valve disposed downstream of the DNA manipulation zone;	<p data-bbox="793 1260 1814 1325">The accused system comprises a microfluidic device comprising a second valve disposed downstream of the DNA manipulation zone</p> <p data-bbox="793 1365 1860 1398"><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6,</p>

Claim	Claim Language	Infringement Evidence
		<p>2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p>The evidence consists of four panels (A, B, C, D) illustrating the operation of a microfluidic cartridge. Panel A shows a pipette tip approaching a valve. Panel B shows the pipette tip dispensing a droplet. Panel C shows the droplet moving through a channel. Panel D shows the droplet entering a chamber. Below these panels is a larger diagram showing a 'Second valve' and a 'PCR' chamber with green droplets.</p>

Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second

Claim	Claim Language	Infringement Evidence
		<p>branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
19(i)	a controller programmed to close the first and second valves to prevent gas and liquid from	The accused system comprises a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the DNA manipulation zone and to isolate and confine the sample to a region between the first and second valves

Claim	Claim Language	Infringement Evidence
	<p>flowing into or out of the DNA manipulation zone and to isolate and confine the sample to a region between the first and second valves accessible to the detector,</p>	<p>accessible to the detector.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.”

Claim	Claim Language	Infringement Evidence
		<p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 24, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> • Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway;

Claim	Claim Language	Infringement Evidence
		<p>capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads.</p> <ul style="list-style-type: none"> • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber. • U.S. Patent No. 9,339,812 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)

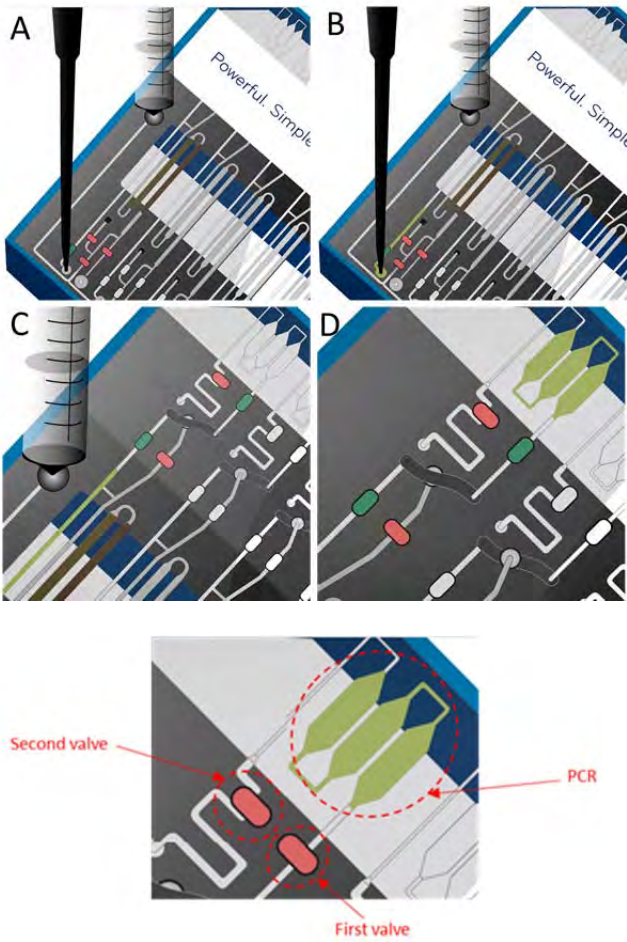
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • U.S. Patent No. 9,339,812 at 3:41-46 (“The detection chamber heaters 157, optical subsystem 180 and valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate analysis of the set of nucleic acid-reagent mixtures by a processor configured to display information on a user interface.”) • U.S. Patent No. 9,339,812 at 26:25-32 (“In a variation wherein the controller 272 is coupled to the molecular diagnostic module 130, the controller 272 preferably functions to automate reception of a microfluidic cartridge, heating of biological samples within the molecular diagnostic module 130 and the detection chambers 213, occlusion of fluidic pathways 220 by the valve actuation subsystem 170, and analysis of a set of nucleic acid-reagent mixtures by the optical subsystem 180.”) • U.S. Patent No. 9,339,812 at 33:3-39 (“Embodiments of the method 400 and variations thereof can be embodied and/or implemented at least in part by a machine configured to receive a computer-readable medium storing computer-readable instructions. The instructions are preferably executed by computer-executable components preferably integrated with the system 100 and one or more portions of the processor 273 and/or the controller 272. The computer-readable medium can be stored on any suitable computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices (CD or DVD), hard drives, floppy drives, or any suitable device. The computer-executable component is preferably a general or application specific processor, but any suitable dedicated hardware or hardware/firmware combination device can alternatively or additionally execute the instructions. The FIGURES illustrate the architecture, functionality and operation of possible implementations of systems, methods and computer program products according to preferred embodiments, example configurations, and variations thereof. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks

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		<p>shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.”)</p> <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined

Claim	Claim Language	Infringement Evidence
		<p>between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • US Patent No. 9,738,887 at 12:11-19 (“When not in operation, however, the normally closed position 43 is configured to prevent leakage and/or fluid bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.”) • US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by

Claim	Claim Language	Infringement Evidence
		<p>using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at Figs. 1J and 1K: <div data-bbox="961 641 1753 933"> <p style="text-align: center;">FIG. 1J</p> </div> <div data-bbox="961 941 1753 1234"> <p style="text-align: center;">FIG. 1K</p> <p style="text-align: right;">○ OCCLUDED</p> </div> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection

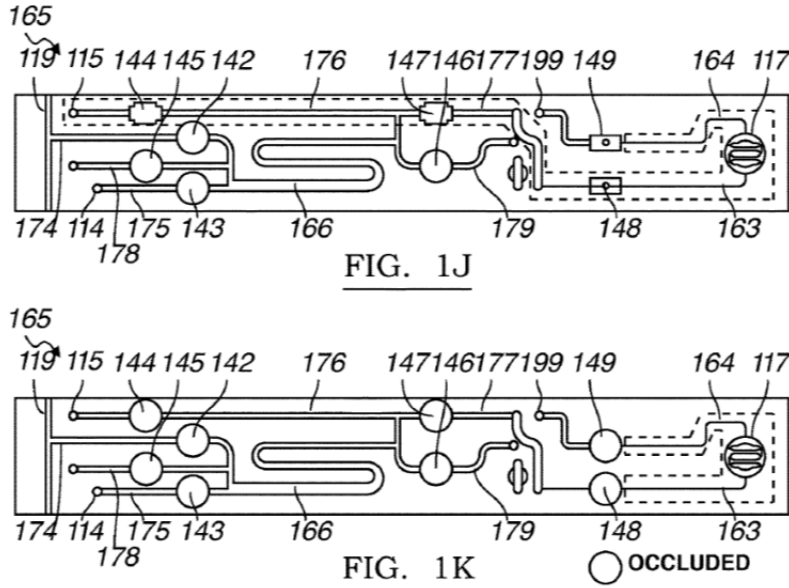
Claim	Claim Language	Infringement Evidence
		chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
19(j)	wherein the only ingress to and egress from the region accessible to the detector is through the first and second valves; and	<p>In the accused system, the only ingress to and egress from the region accessible to the detector is through the first and second valves.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

Claim	Claim Language	Infringement Evidence
		 <p>The diagram consists of four panels labeled A, B, C, and D, illustrating the operation of a microfluidic cartridge. Panels A and B show a pipette tip dispensing liquid into a well of the cartridge. Panels C and D show the internal fluidic pathways, including valves and chambers. A detailed inset below shows a close-up of the internal components, with labels for 'Second valve', 'First valve', and 'PCR' (polymerase chain reaction) chambers. The text 'Powerful. Simple.' is visible on the top right of panels A and B.</p> <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module,

Claim	Claim Language	Infringement Evidence
		<p>the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads.</p> <ul style="list-style-type: none"> • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber. <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve

Claim	Claim Language	Infringement Evidence
		<p>guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
19(k)	wherein the computer-controlled heat source is in thermal contact with the DNA manipulation zone and	<p>The accused system comprises a computer-controlled heat source in thermal contact with the DNA manipulation zone.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/,</p>

Claim	Claim Language	Infringement Evidence
		<p>last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO </p>

Claim	Claim Language	Infringement Evidence
		<p>NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, last visited May 31, 2019, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> • “There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.” • “The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate

Claim	Claim Language	Infringement Evidence
		<p>connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled

Claim	Claim Language	Infringement Evidence
		<p>to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:21-32 (“As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection 30 chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.”) • U.S. Patent No. 9,499,896 at 9:11-19 (“As shown in FIGS. 1, 4A-4B, and 7A-7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heater-sensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the system 100, a set of elastic elements 160 configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.”) • U.S. Patent No. 9,499,896 at 12:20-31 (“In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistance-to-voltage

Claim	Claim Language	Infringement Evidence
		<p>conversion circuit because the UT750 PID controller requires voltage as an input for PID control.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 11:63-12:4 “As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100.”
19(l)	wherein the detector is configured to identify one or more polynucleotides within the DNA manipulation zone.	<p>The accused system comprises a detector configured to identify one or more polynucleotides within the DNA manipulation zone.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019 (Exhibit 14)</p> <ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p>JFO_2018-10-25_8009-Rev-B_NeuMoDx-96-Spec-Sheet (Exhibit 21)</p>


Claim	Claim Language	Infringement Evidence																																				
		<table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>NeuMoDx_288_Spec_Sheet_R2.pdf (Exhibit 22)</p> <table> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </table> <p>US10041062 (Exhibit 33)</p> <ul style="list-style-type: none"> Claim 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising: a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode; a nozzle of a liquid handling subsystem; an optical subsystem; a cartridge heater; a magnet vertically aligned with the magnet receiving slot; and an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein: the nozzle of the liquid handling system is coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample, the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge, the optical subsystem interfaces with a second portion 	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
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Claim	Claim Language	Infringement Evidence
		<p>of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and a third region of the cartridge is compressed between the cartridge heater and the cartridge platform.</p> <ul style="list-style-type: none"> Claim 8. The system of claim 1, wherein the optical subsystem comprises at least one unit including an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the biological sample, and to transmit emitted light from the biological sample, through the emission filter, and toward the photodetector. <p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater. Claim 11. The system of claim 1, wherein the molecular diagnostic module further comprises an optical subsystem comprising a first unit and a second unit, wherein each of the first unit and the second unit includes a set of excitation filters, a set of emission filters, a set of photodetectors aligned

Claim	Claim Language	Infringement Evidence
		<p>with the set of emission filters, and a set of dichroic mirrors configured to reflect light from the set of excitation filters toward one of a set of nucleic acid-reagent mixtures at the cartridge, and to transmit emitted light from one of the set of nucleic acid-reagent mixtures, through at least one of the set of emission filters, and toward at least one of the set of photodetectors.</p> <ul style="list-style-type: none"> • Claim 12. The system of claim 11, wherein the molecular diagnostic module further includes a set of detection chamber heaters configured to heat a set of detection chambers through the second surface of the cartridge, and wherein the optical subsystem is configured to receive light, emitted from the set of nucleic acid-reagent mixtures at the set of detection chambers, from the first surface of the cartridge.

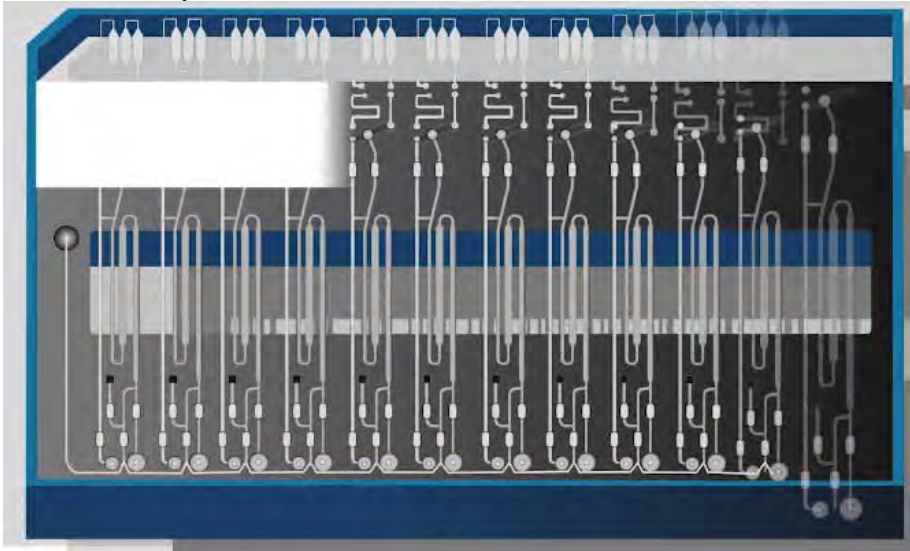
Exhibit 35

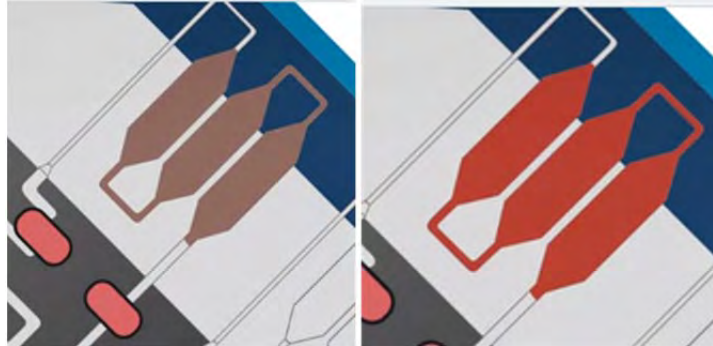
U.S. Patent No. 8,703,069 Infringement Chart

Claim	Claim Language	Infringement Evidence
1(a)	1. A method of amplifying a nucleic acid-containing sample within a microfluidic device, the method comprising:	<p>To the extent the preamble is limiting, the accused workflow is a method of amplifying a nucleic acid-containing sample within a microfluidic device.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.”

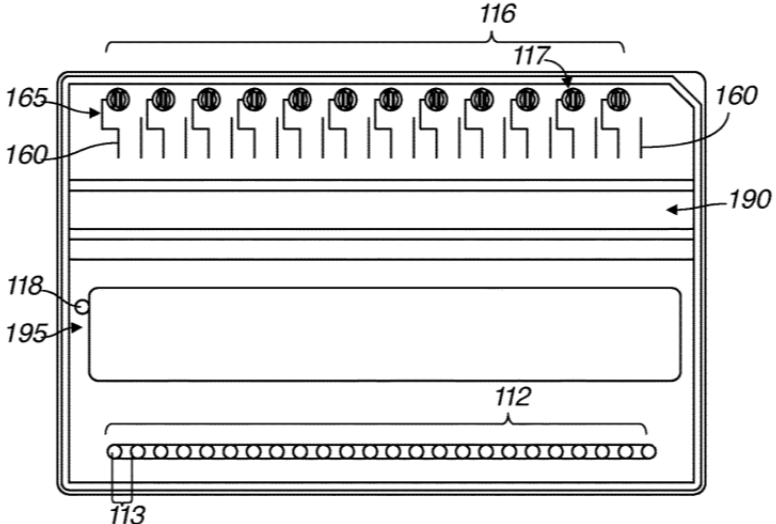
Claim	Claim Language	Infringement Evidence
		<p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</p> <ul style="list-style-type: none"> • “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019 (Exhibit 14)</p> <ul style="list-style-type: none"> • “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of

Claim	Claim Language	Infringement Evidence
		<p>products of amplification.”</p> <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>0600101_Rev-D-IFU-NeuMoDx-RELEASE-Solution-US-ONLY-FINAL-25Oct2018.pdf (Exhibit 20)</p> <ul style="list-style-type: none"> • “NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE... The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) and the dried Master Mix contained in a NeuMoDx test strip. The System then dispenses the prepared RT-PCR-ready mixture into the NeuMoDx Cartridge where Real-Time PCR occurs.” <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> • “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

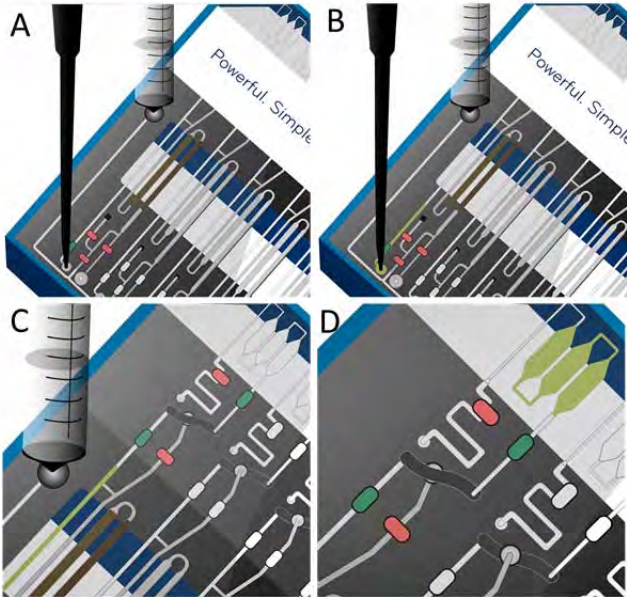
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> <li data-bbox="842 238 1898 342">• “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> <li data-bbox="842 899 1856 1003">• “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 <li data-bbox="842 1013 1919 1224">• “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

Claim	Claim Language	Infringement Evidence										
		<div></div> <p>“Patents”, http://www.neumodx.com/patents/, demonstrating that NeuMoDx marks its products with US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; 9,452,430; 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; 10,010,888; 9,382,532; 9,540,636; 9,499,896; 9,539,576; 9,637,775; and 10,093,963. (Exhibit 15)</p> <h2>PATENTS</h2> <table><tr><th>Product</th><th>Patents</th></tr><tr><td>CARTRIDGE</td><td>US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.</td></tr><tr><td>P02 (overall system and method)</td><td>US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.</td></tr><tr><td>EXTRACTION PLATE</td><td>US Patent Nos. 9,382,532; and 9,540,636.</td></tr><tr><td>XPCR MODULE</td><td>US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.</td></tr></table> <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none">Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate	Product	Patents	CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.	P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.	EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.	XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.
Product	Patents											
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Claim	Claim Language	Infringement Evidence
		<p>substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent port, the fluid port, and the detection chamber.</p> <ul style="list-style-type: none"> • Claim 11. The cartridge of claim 1, wherein the detection chamber comprises a first, a second, and a third detection chamber segment wherein each of the first, the second, and the third detection chamber segment is a broad chamber of which a projection onto a plane is substantially rectangular, wherein a first end of the second detection chamber segment is connected to the first detection chamber segment by a first narrow fluidic channel, and wherein a second end of the second detection chamber segment is connected to the third detection chamber segment by a second narrow fluidic channel. • U.S. Patent No. 9,738,887 at FIG. 1A:

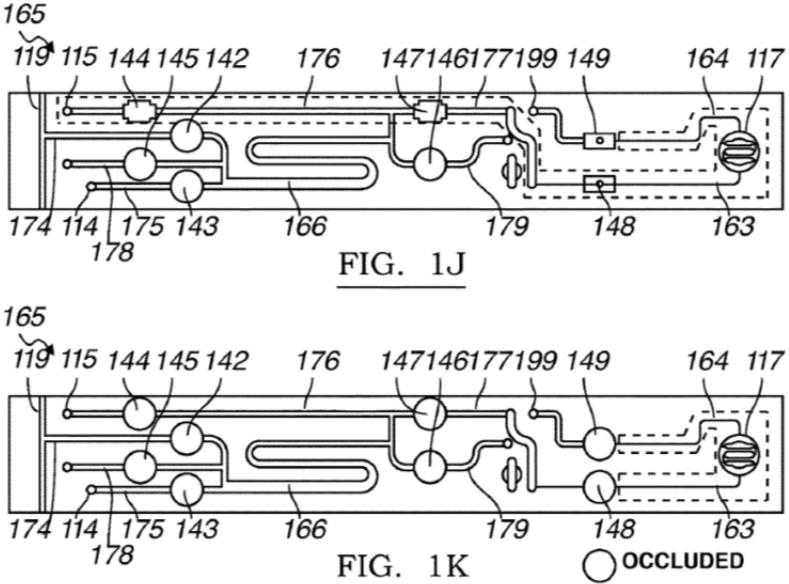
Claim	Claim Language	Infringement Evidence
		 <p style="text-align: center;">FIG. 1A</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at Abstract (“A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region.”) US Patent No. 9,738,887 at 2:36-3:5. (“As shown in FIGS. 1A-1C, an embodiment of a microfluidic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample port-

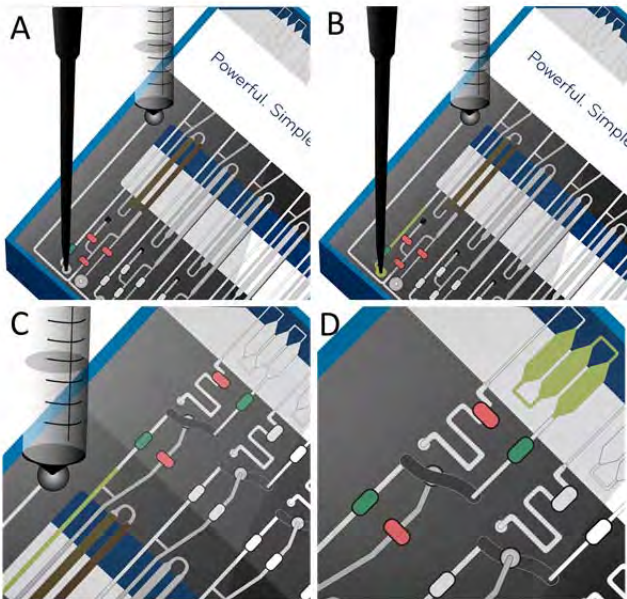
Claim	Claim Language	Infringement Evidence
		<p>reagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; and a set of fluidic pathways 160, each formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140... In a specific application, the microfluidic cartridge 100 can be used to facilitate a PCR procedure for analysis of a sample containing nucleic acids.")</p> <ul style="list-style-type: none"> • US Patent No. 9,738,887 at 13:7-18. ("The top layer 110 of an embodiment of the microfluidic cartridge 100 functions to accommodate elements involved in performing a molecular diagnostic procedure (e.g. PCR), such that a sample containing nucleic acids, passing through the cartridge, can be manipulated by the elements involved in performing the molecular diagnostic procedure. The top layer 110 is preferably composed of a structurally rigid/stiff material with low autofluorescence, such that the top layer 110 does not interfere with sample detection by fluorescence or chemiluminescence techniques, and an appropriate glass transition temperature and chemical compatibility for PCR or other amplification techniques.") • US Patent No. 9,738,887 at 13:35-42. ("The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.") • US Patent No. 9,738,887 at 15:29-39 ("The segments may be arranged in at least one of several configurations to facilitate isolation, processing, and amplification of a nucleic acid sample ..."). • US Patent No. 9,738,887 at 23:20-24 ("The top layer 110 of the specific embodiment of the microfluidic cartridge 100 functions preferably as described

Claim	Claim Language	Infringement Evidence
		<p>in Section 1.1, and is composed of polypropylene with low autofluorescence and a glass transition temperature suitable for PCR.")</p>
1(b)	<p>moving the sample from an upstream channel of the microfluidic device into a DNA manipulation module located downstream of the upstream channel,</p>	<p>The accused workflow includes moving the sample from an upstream channel of the microfluidic device into a DNA manipulation module located downstream of the upstream channel.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to "VIDEO NeuMoDx™ WORKFLOW" hyperlink at https://player.vimeo.com/video/299307936. (<u>Exhibit 16</u>)</p> <ul style="list-style-type: none"> • "A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins." <i>Id.</i> at 3:58-4:08 

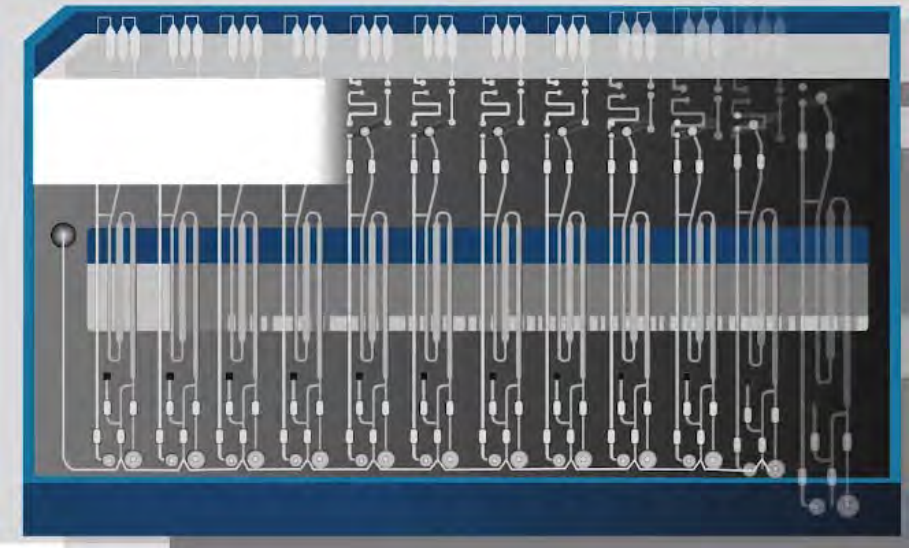
Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion

Claim	Claim Language	Infringement Evidence
		<p>positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • US Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • US Patent No. 9,738,887 at 15:31-35 (“The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.”) • US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid

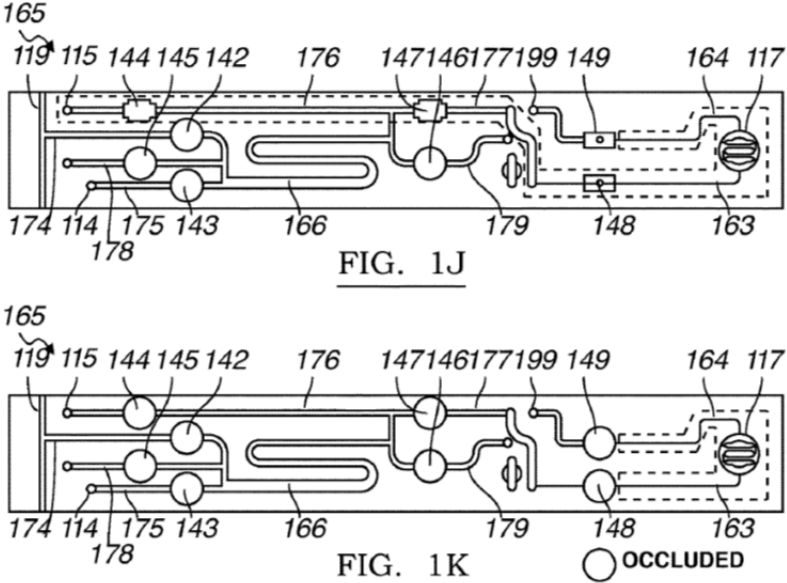
Claim	Claim Language	Infringement Evidence
		<p>sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at Figs. 1J and 1K:  <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p>
1(c)	the DNA manipulation module including a DNA manipulation zone configured to perform amplification of the sample,	<p>The accused workflow includes a DNA manipulation module including a DNA manipulation zone configured to perform amplification of the sample.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

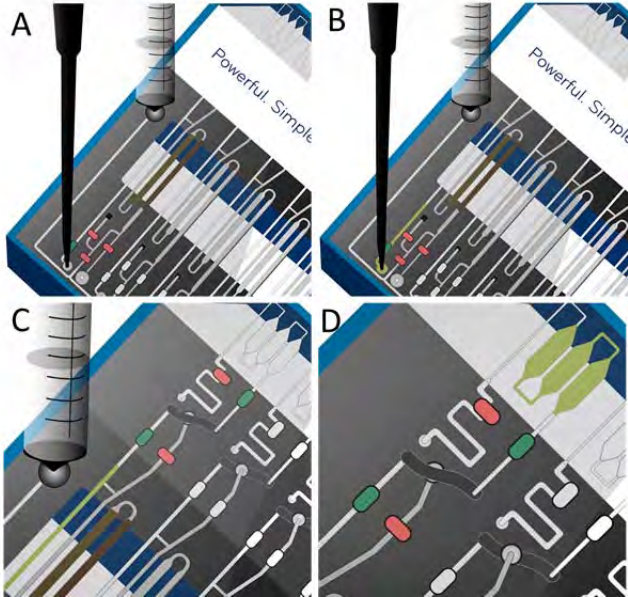
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “<i>NeuMoDx™ 288</i> and <i>NeuMoDx™ 96</i> Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 24, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “The <i>NeuMoDx™</i> Molecular Systems are a family of scalable platforms that

Claim	Claim Language	Infringement Evidence
		<p>fully integrate the entire molecular diagnostic process from ‘sample to result’. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.”</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>0600101_Rev-D-IFU-NeuMoDx-RELEASE-Solution-US-ONLY-FINAL-25Oct2018.pdf (Exhibit 20)</p> <ul style="list-style-type: none"> • “NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE... The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) and the dried Master Mix contained in a NeuMoDx test strip. The System then dispenses the prepared RT-PCR-ready mixture into the NeuMoDx Cartridge where Real-Time PCR occurs.”

Claim	Claim Language	Infringement Evidence
		<p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.”

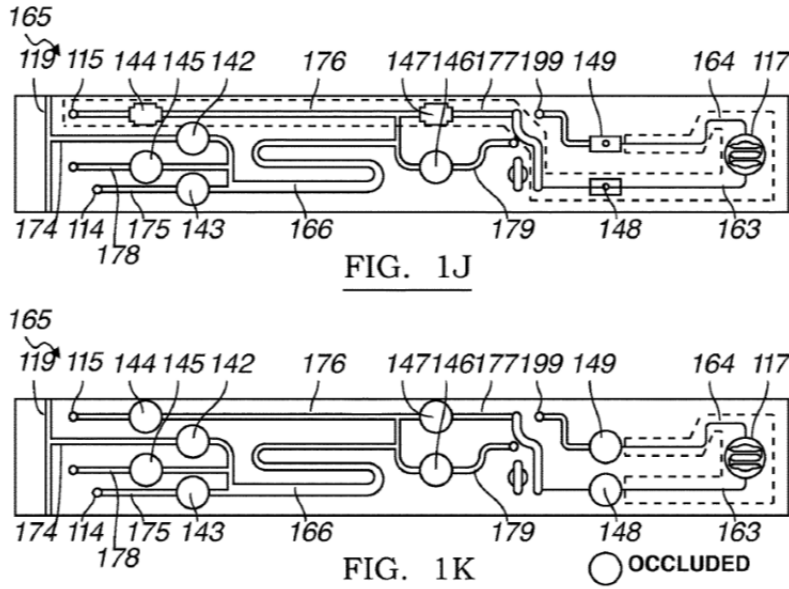
Claim	Claim Language	Infringement Evidence
		<p><i>Id.</i> at 3:58-4:08</p> <p>U.S. Patent No. 9,738,887</p> <ul style="list-style-type: none"> Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent port, the fluid port, and the detection chamber. U.S. Patent No. 9,738,887 at Abstract (“A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region”) <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which

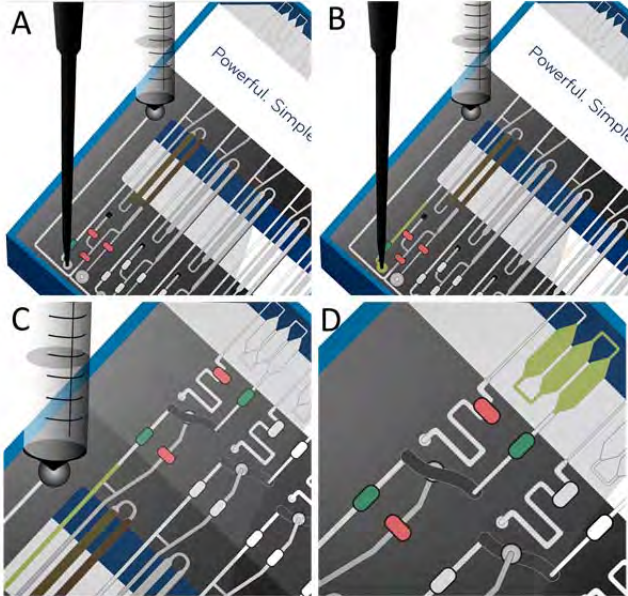
Claim	Claim Language	Infringement Evidence
		<p>volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.")</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 17:27-49 ("Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115.... An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.") U.S. Patent No. 9,738,887 at Figs. 1J and 1K:  <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p>

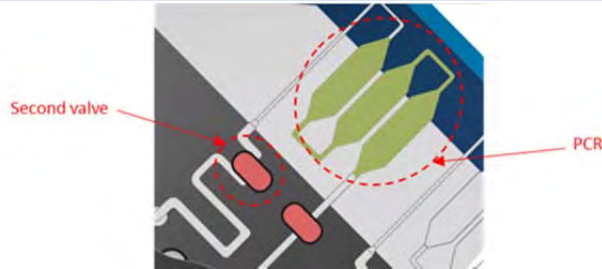
Claim	Claim Language	Infringement Evidence
1(d)	a first valve disposed upstream of the DNA manipulation zone,	<p>The accused workflow includes a first valve disposed upstream of the DNA manipulation zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (<u>Exhibit 16</u>)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 

Claim	Claim Language	Infringement Evidence
		<div data-bbox="1079 228 1528 532" data-label="Image"> </div> <p data-bbox="793 613 1115 646">US9738887 (Exhibit 31)</p> <ul data-bbox="842 654 1919 1414" style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second

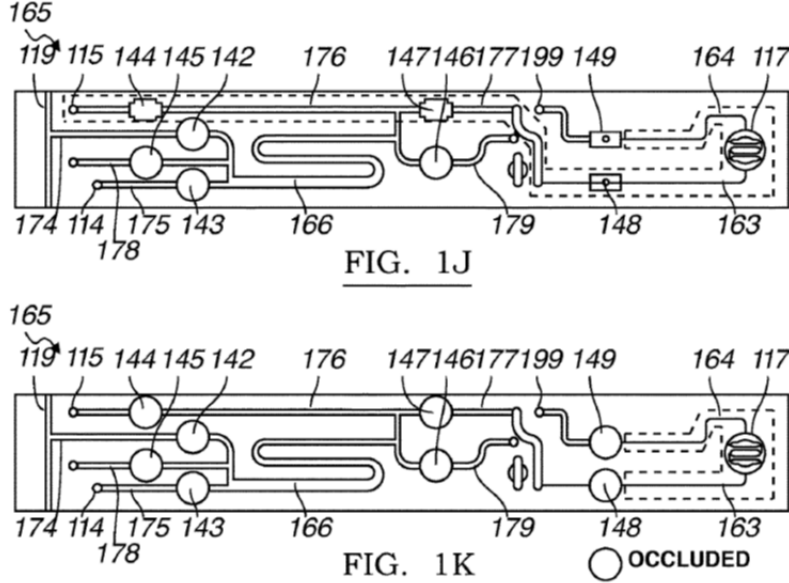
Claim	Claim Language	Infringement Evidence
		<p>surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured

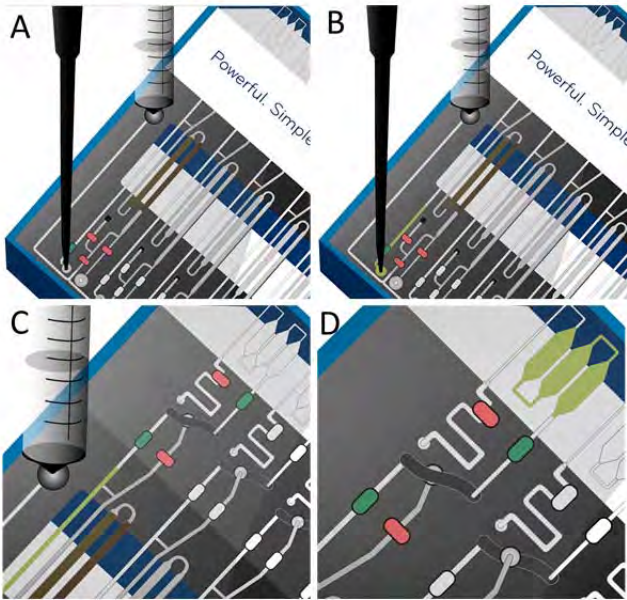
Claim	Claim Language	Infringement Evidence
		<p>to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)at Figs. 1J and 1K:</p>  <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)

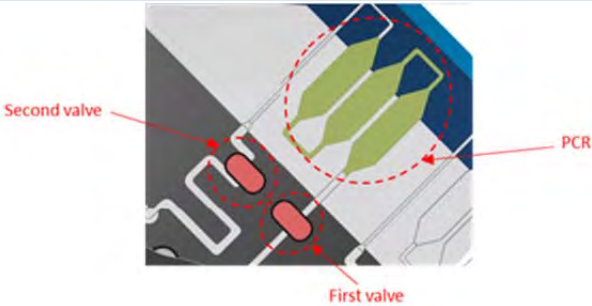
Claim	Claim Language	Infringement Evidence
1(e)	and a second valve disposed downstream of the DNA manipulation zone,	<p>The accused workflow includes a second valve disposed downstream of the DNA manipulation zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 

Claim	Claim Language	Infringement Evidence
		 <p>The diagram shows a top-down view of a microfluidic device. A red dashed circle highlights a region containing two red oval-shaped components labeled 'Second valve' and a green, elongated, multi-lobed structure labeled 'PCR'. The device features a network of white channels on a grey substrate.</p> <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a

Claim	Claim Language	Infringement Evidence
		<p>closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic

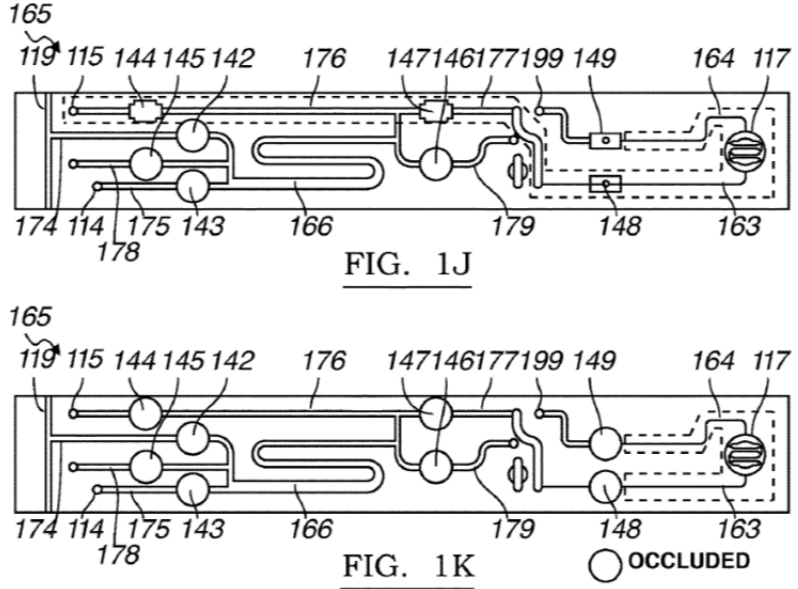
Claim	Claim Language	Infringement Evidence
		<p>system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) at Figs. 1J and 1K:</p>  <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
1(f)	the only ingress to and egress	In the accused workflow, the only ingress to and egress from the DNA manipulation

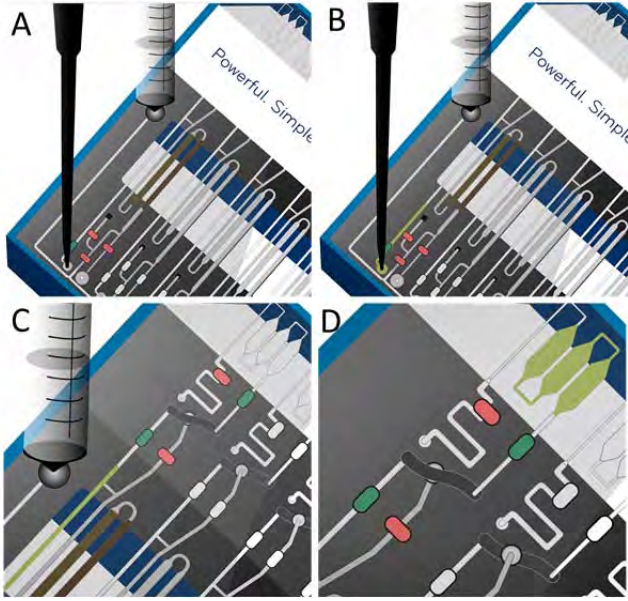
Claim	Claim Language	Infringement Evidence
	from the DNA manipulation zone being through the first valve and the second valve;	<p>zone being through the first valve and the second valve.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 

Claim	Claim Language	Infringement Evidence
		 <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> • Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads. • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to

Claim	Claim Language	Infringement Evidence
		<p>facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber.</p> <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second

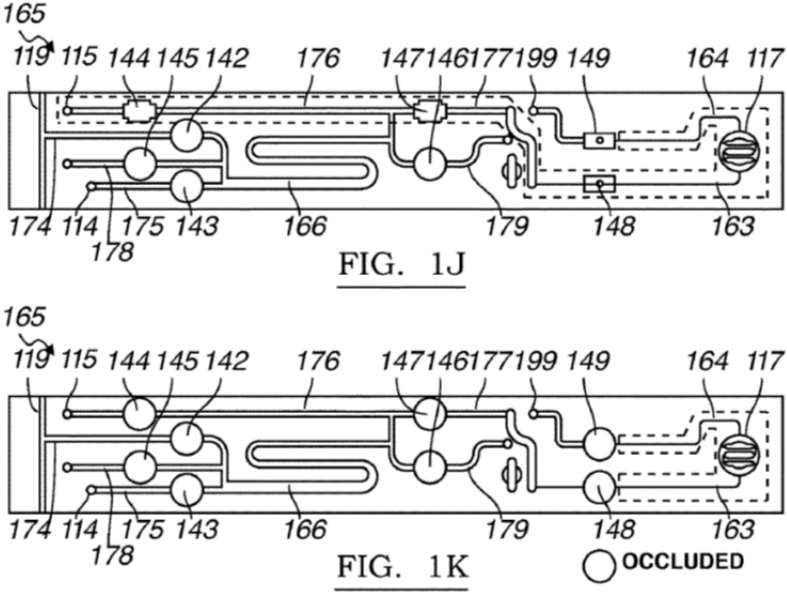
Claim	Claim Language	Infringement Evidence
		<p>surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144,

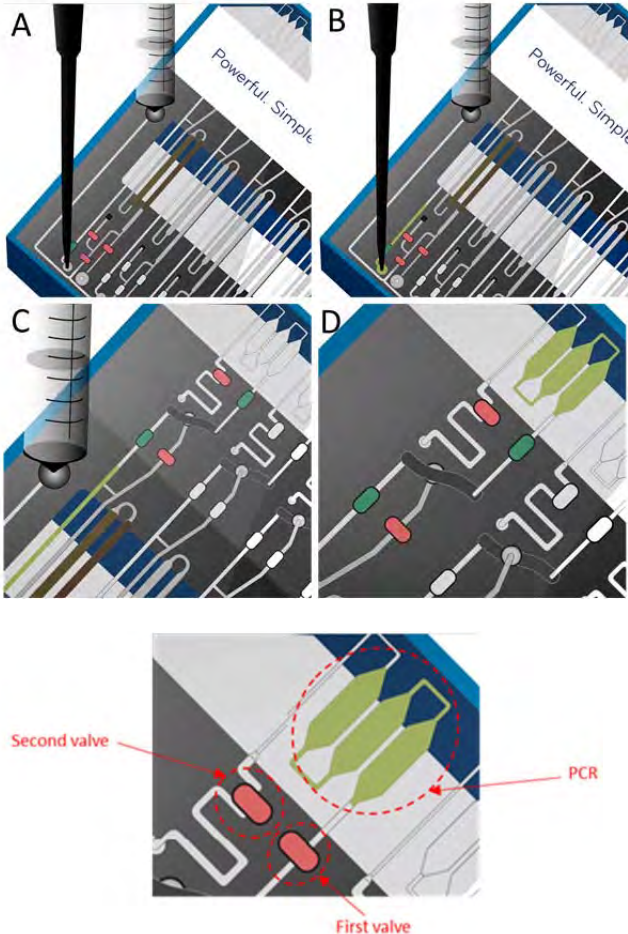
Claim	Claim Language	Infringement Evidence
		<p>147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)at Figs. 1J and 1K:</p>  <p>FIG. 1J</p> <p>FIG. 1K ○ OCCLUDED</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as

Claim	Claim Language	Infringement Evidence
		shown in FIG. 1C.”)
1(g)	receiving the sample in the DNA manipulation zone;	<p>The accused workflow includes receiving the sample in the DNA manipulation zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (<u>Exhibit 16</u>)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection

Claim	Claim Language	Infringement Evidence
		<p>chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p>

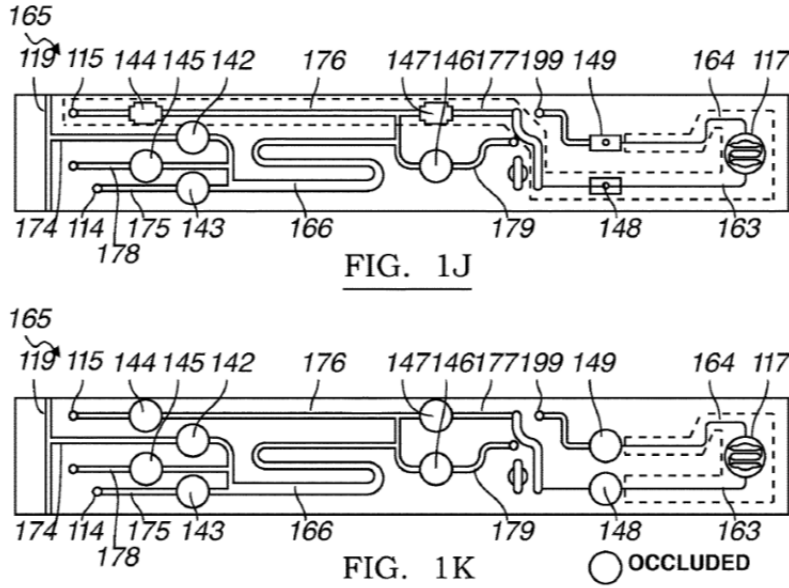
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • US Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • US Patent No. 9,738,887 at 15:31-35 (“The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.”) • US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic

Claim	Claim Language	Infringement Evidence
		<p>pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at Figs. 1J and 1K:  <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p>
1(h)	closing the first valve and the second valve such that gas and liquid are prevented from flowing into or out of the DNA manipulation zone; and	<p>The accused workflow includes closing the first valve and the second valve such that gas and liquid are prevented from flowing into or out of the DNA manipulation zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process

Claim	Claim Language	Infringement Evidence
		<p data-bbox="890 235 1203 264">begins.” <i>Id.</i> at 3:58-4:08</p>  <p data-bbox="793 1289 1113 1318">US9738887 (Exhibit 31)</p> <ul data-bbox="846 1328 1898 1396" style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection

Claim	Claim Language	Infringement Evidence
		<p>chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> US Patent No. 9,738,887 at 12:11-19 (“When not in operation, however, the normally closed position 43 is configured to prevent leakage and/or fluid bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.”) US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) US Patent No. 9,738,887 at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
1(i)	thermal cycling the sample in the DNA manipulation zone.	<p>The accused workflow includes thermal cycling the sample in the DNA manipulation zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6,</p>

Claim	Claim Language	Infringement Evidence
		<p>2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 • “During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26 <div data-bbox="905 634 1612 980"> </div> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 24, 2019 (Exhibit 11)</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>0600101_Rev-D-IFU-NeuMoDx-RELEASE-Solution-US-ONLY-FINAL-25Oct2018.pdf (Exhibit 20)</p> <ul style="list-style-type: none"> • “NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE... The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) and the dried Master Mix contained in a NeuMoDx test strip. The System then dispenses the prepared RT-PCR-ready mixture into the NeuMoDx Cartridge where Real-Time PCR occurs.” <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> • “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> • A system for thermocycling biological samples within detection chambers


Claim	Claim Language	Infringement Evidence
		<p>comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <p>US9101930 (Exhibit 25)</p> <ul style="list-style-type: none"> Claim 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising: a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer; a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, are each at least partially separated from the corrugated surface of the intermediate substrate by

Claim	Claim Language	Infringement Evidence
		<p>an elastomeric layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate.</p> <ul style="list-style-type: none"> • Claim 11. The cartridge of claim 10, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. • Claim 22. The cartridge of claim 11, wherein at least one of the first detection chamber and the second detection chamber is configured to be optimized for volumetric capacity, thermocycling rates, optical detection, and filling in a manner that limits bubble generation. <p>US9604213 (Exhibit 30)</p> <ul style="list-style-type: none"> • Claim 1. A system for processing and detecting nucleic acids in cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising: a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising: a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation, a heater configured to transmit heat to the heating region at the first surface during operation, and a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during

Claim	Claim Language	Infringement Evidence
		<p>operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater.</p> <ul style="list-style-type: none"> • Claim 11. The system of claim 1, wherein the molecular diagnostic module further comprises an optical subsystem comprising a first unit and a second unit, wherein each of the first unit and the second unit includes a set of excitation filters, a set of emission filters, a set of photodetectors aligned with the set of emission filters, and a set of dichroic mirrors configured to reflect light from the set of excitation filters toward one of a set of nucleic acid-reagent mixtures at the cartridge, and to transmit emitted light from one of the set of nucleic acid-reagent mixtures, through at least one of the set of emission filters, and toward at least one of the set of photodetectors. • Claim 12. The system of claim 11, wherein the molecular diagnostic module further includes a set of detection chamber heaters configured to heat a set of detection chambers through the second surface of the cartridge, and wherein the optical subsystem is configured to receive light, emitted from the set of nucleic acid-reagent mixtures at the set of detection chambers, from the first surface of the cartridge.


EXHIBIT 36

U.S. Patent No. 7,998,708 Infringement Chart

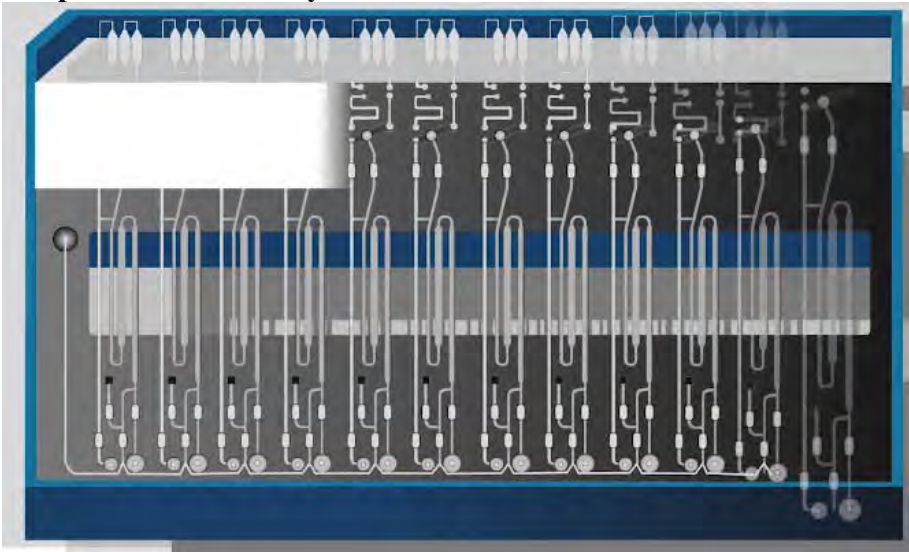
Claim	Claim Language	Infringement Evidence
1(a)	An apparatus, comprising:	<p>To the extent the preamble is limiting, the accused instruments are an apparatus.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p>  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited</p>

Claim	Claim Language	Infringement Evidence
		<p>May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of

Claim	Claim Language	Infringement Evidence
		<p>the instrument with touchscreen computer, accessories, and reagents and consumables.”</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/dr-steven-young-video-testimonial/, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> At 2:58-3:18 (“There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.”)
1(b)	a multi-lane microfluidic cartridge, each lane comprising a PCR reaction zone;	<p>The accused system comprises a multi-lane microfluidic cartridge, each lane comprising a PCR reaction zone.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

Claim	Claim Language	Infringement Evidence
		 <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized

Claim	Claim Language	Infringement Evidence
		<p>reagents.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> <li data-bbox="846 237 1892 342">• “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> <li data-bbox="846 898 1892 1003">• “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 <p data-bbox="793 1044 1892 1187">“Patents”, http://www.neumodx.com/patents/, demonstrating that NeuMoDx marks its products with US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; 9,452,430; 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; 10,010,888; 9,382,532; 9,540,636; 9,499,896; 9,539,576; 9,637,775; and 10,093,963 (Exhibit 15)</p>


Claim	Claim Language	Infringement Evidence										
		<div>PATENTS</div> <table><tr><th>Product</th><th>Patents</th></tr><tr><td>CARTRIDGE</td><td>US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.</td></tr><tr><td>P02 (overall system and method)</td><td>US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.</td></tr><tr><td>EXTRACTION PLATE</td><td>US Patent Nos. 9,382,532; and 9,540,636.</td></tr><tr><td>XPCR MODULE</td><td>US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.</td></tr></table> <div>US9403165 (Exhibit 27)<ul style="list-style-type: none">Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate.Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic</div>	Product	Patents	CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.	P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.	EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.	XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.
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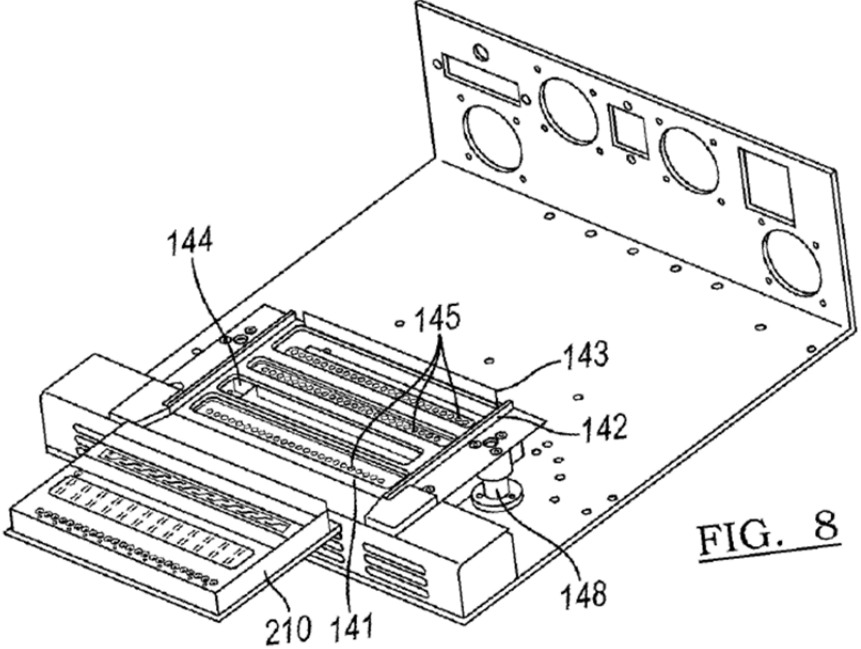
Claim	Claim Language	Infringement Evidence
		<p>pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to

Claim	Claim Language	Infringement Evidence
		<p>produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a

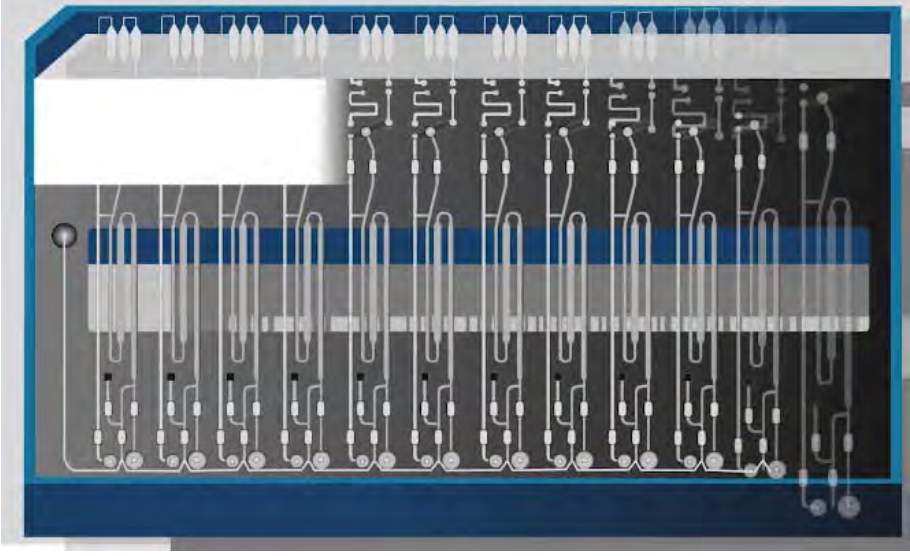
Claim	Claim Language	Infringement Evidence
		<p>capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent

Claim	Claim Language	Infringement Evidence
		<p>mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)</p>
1(c)	a receiving bay configured to receive the microfluidic cartridge;	<p>The accused system comprises a receiving bay configured to receive the microfluidic cartridge.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ TECHNOLOGY” hyperlink at https://player.vimeo.com/video/281470603. (Exhibit 17)</p> <ul style="list-style-type: none"> • at 4:55-5:00

Claim	Claim Language	Infringement Evidence
		  <p data-bbox="793 1122 1115 1159">US9050594 (Exhibit 24)</p> <ul data-bbox="842 1166 1919 1414" style="list-style-type: none"> • Claim 1. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator

Claim	Claim Language	Infringement Evidence
		<p>configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 2:6-7 (“FIG. 8 depicts an embodiment of a microfluidic cartridge and an embodiment of a cartridge platform.”) • U.S. Patent No. 9,050,594 at Fig. 8  <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 7:53-8:35 “As shown in FIG. 9A, the cartridge receiving module 140 of the molecular diagnostic module 130 comprises a cartridge platform 141 including a cartridge loading guiderail 142, a cartridge

Claim	Claim Language	Infringement Evidence
		<p>stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145; a linear actuator 146 configured to displace a microfluidic cartridge 210 resting on the cartridge platform 141, and a set of springs 148 coupled to the cartridge platform 141. The cartridge receiving module 140 thus functions to receive, align, and compress a microfluidic cartridge 210 for processing of a biological sample according to a molecular diagnostic assay protocol.... The cartridge platform 141 includes a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145, and functions to receive and align a microfluidic cartridge 210, while providing access to the microfluidic cartridge 210 by a magnet 160 and a valve actuation subsystem 170. As shown in FIG. 8, an embodiment of the cartridge platform 141 includes a pair of parallel cartridge loading guiderails 142, initiating at a pair of inwardly tapering protrusions configured to guide a microfluidic cartridge toward the pair of parallel cartridge loading guiderails 142, and spanning two short edges of the cartridge platform 141. The embodiment of the cartridge platform 141 also includes a cartridge stop 143 comprising a vertical tab oriented perpendicular to the cartridge loading guiderails 142, and spanning a long edge of the cartridge platform. Preferably, the cartridge loading guiderails 142 and the cartridge stop 143 are configured such that a microfluidic cartridge 210 slides between the cartridge loading guiderails 142 and hits the cartridge stop 143 to signal proper alignment.”</p>
1(d)	<p>each PCR reaction zone comprising a separately controllable heat source thermally coupled thereto, wherein the heat source maintains a substantially uniform temperature throughout the PCR reaction zone and thermal cycles the PCR reaction zone to carry out PCR on a polynucleotide-containing</p>	<p>The accused system comprises a multi-lane microfluidic cartridge, each lane comprising a PCR reaction zone and each PCR reaction zone comprising a separately controllable heat source thermally coupled thereto, wherein the heat source maintains a substantially uniform temperature throughout the PCR reaction zone and thermal cycles the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936.</p>

Claim	Claim Language	Infringement Evidence
	sample in the PCR reaction zone;	<p>(Exhibit 16)</p> <ul style="list-style-type: none"> <p>“This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59</p>  <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p>

Claim	Claim Language	Infringement Evidence
		<div data-bbox="905 233 1612 574" data-label="Image"> </div> <p data-bbox="793 613 1877 683"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="842 695 1919 1019" style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p data-bbox="793 1057 1108 1089">US9050594 (Exhibit 24)</p> <ul data-bbox="842 1101 1919 1421" style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet

Claim	Claim Language	Infringement Evidence
		<p>receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module

Claim	Claim Language	Infringement Evidence
		<p>comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter.</p> <ul style="list-style-type: none"> • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic

Claim	Claim Language	Infringement Evidence
		<p>pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers. U.S. Patent No. 9,499,896 at 2:33-48 “The system 100 functions to enable

Claim	Claim Language	Infringement Evidence
		<p>rapid thermal cycling of samples while providing uniform heating and preventing signal drift. In specific applications, the system 100 can be used to rapidly and controllably thermocycle nucleic acid samples during performance of molecular diagnostic amplification techniques (e.g., PCR, RT-PCR), signal amplification techniques (e.g., bDNA, hybrid capture), and analytical techniques (e.g., gel electrophoresis, mass spectrometry). The system 100 can also provide rapid thermocycling without significant power requirements, ensure a closer correlation between the actual heating temperature and the temperature set-point by implementing an integrated heater-sensor die, and controllably and individually heat small sample volumes (e.g., picoliters, nanoliters) based upon a microfabrication technique that also enables mass production of the system 100.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:61-3:3 (“The set of heater-sensor dies 110 functions to controllably heat individual sample volumes. Preferably, each heater sensor die 111 is a thin-film die that can be deposited onto another substrate (e.g., silicon, glass substrate) that can be packaged onto an electronics substrate 140 (e.g., printed circuit board, PCB); however, each heater-sensor die 111 can alternatively comprise any suitable geometry and/or configuration that enables controlled, uniform, and rapid heating of a detection chamber in thermal communication with the heater-sensor die 111.”) • U.S. Patent No. 9,499,896 at 3:23-27 (“Preferably, each heater-sensor die 111 in the set of heater sensor dies 110 comprises an assembly including: a first insulating layer 112a that functions to provide an insulating barrier to isolate the heaters and sensors and a heating region 113 that functions to provide uniform sample heating.”) • U.S. Patent No. 9,499,896 at 12:15-20 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies 110.”)

Claim	Claim Language	Infringement Evidence
		<p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points. U.S. Patent No. 9,539,576 at 9:8-12 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor

Claim	Claim Language	Infringement Evidence
		<p>dies 111 in the set of heater-sensor dies no.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,539,576 at 12:59-64 (“Upon completion of Block S240, individual heater-sensor dies of the set of heater-sensor dies can be coupled to one or multiple electronics substrates in order to provide uniform heating of individual sample containers with independent control of heating parameters provided at each of the set of heater-sensor dies.”)
1(e)	a detector configured to detect the presence of an amplification product in the respective PCR reaction zone; and	<p>The accused system comprises a detector configured to detect the presence of an amplification product in the respective PCR reaction zone.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by

Claim	Claim Language	Infringement Evidence																		
		<p>fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.”</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</p> <ul style="list-style-type: none"> • “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019 (Exhibit 14)</p> <ul style="list-style-type: none"> • “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p>JFO_2018-10-25_8009-Rev-B_NeuMoDx-96-Spec-Sheet (Exhibit 21)</p> <table border="1"> <thead> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> </thead> <tbody> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </tbody> </table>	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
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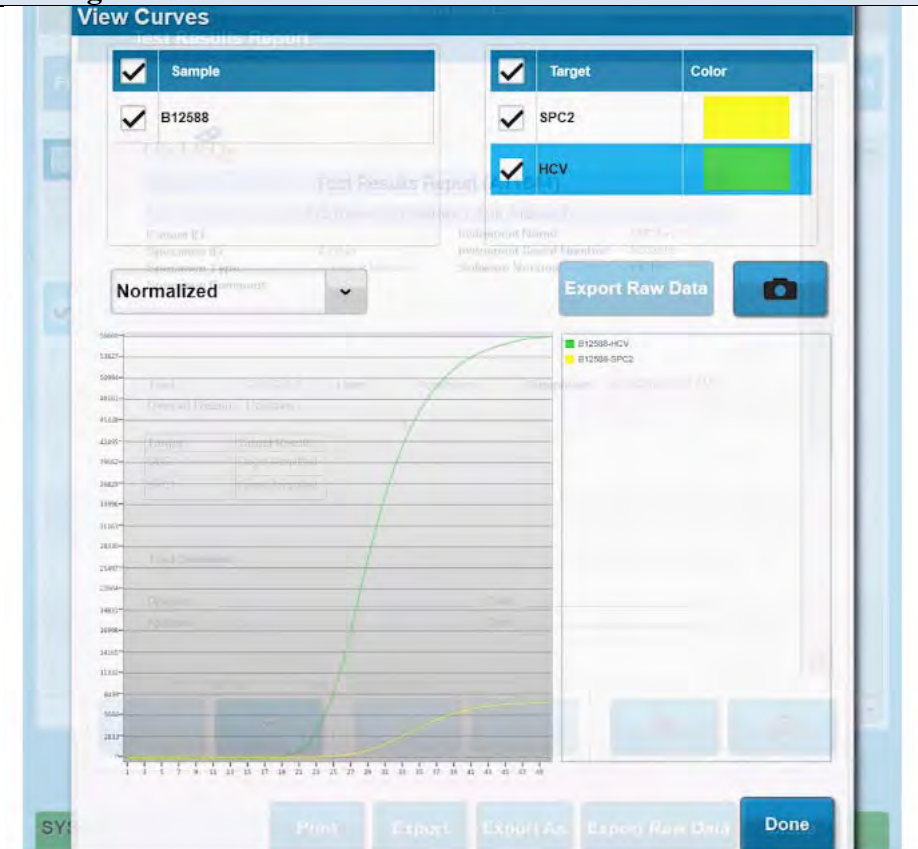
Claim	Claim Language	Infringement Evidence																		
		<p>NeuMoDx_288_Spec_Sheet_R2.pdf (Exhibit 22)</p> <table border="1"> <thead> <tr> <th>Optical Wavelengths</th><th>Excitation (nm)</th><th>Emission (nm)</th></tr> </thead> <tbody> <tr> <td>1</td><td>470</td><td>510</td></tr> <tr> <td>2</td><td>530</td><td>555</td></tr> <tr> <td>3</td><td>585</td><td>610</td></tr> <tr> <td>4</td><td>625</td><td>660</td></tr> <tr> <td>5</td><td>680</td><td>715 long pass</td></tr> </tbody> </table> <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. 	Optical Wavelengths	Excitation (nm)	Emission (nm)	1	470	510	2	530	555	3	585	610	4	625	660	5	680	715 long pass
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Claim	Claim Language	Infringement Evidence
		<p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic

Claim	Claim Language	Infringement Evidence
		<p>acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12

Claim	Claim Language	Infringement Evidence
		<p>different pathways for sample processing.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)
1(f)	a processor coupled to the detector and the heat source, configured to control heating of one or more PCR reaction zones by the heat sources.	<p>The accused system comprises a processor coupled to the detector and the heat source, configured to control heating of one or more PCR reaction zones by the heat sources.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieveing [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a


Claim	Claim Language	Infringement Evidence
		<p>platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

Claim	Claim Language	Infringement Evidence
		 <p><i>NeuMoDxTM Molecular Systems</i>, NEUMODx, http://www.neumodx.com/dr-steven-young-video-testimonial/, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> At 2:58-3:18 (“There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller

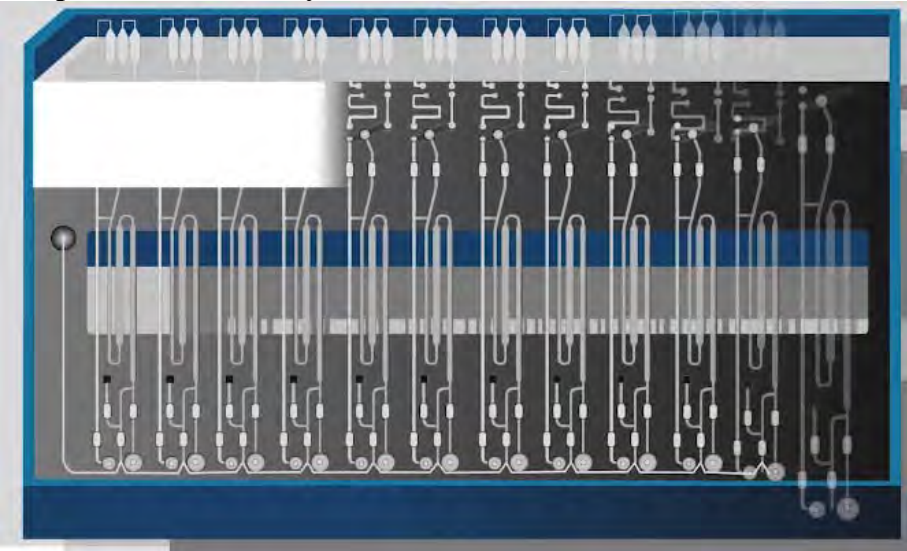
Claim	Claim Language	Infringement Evidence
		<p>footprint.”)</p> <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.

Claim	Claim Language	Infringement Evidence
		<p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers. U.S. Patent No. 9,499,896 at 2:21-32 (“As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection 30 chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.”) U.S. Patent No. 9,499,896 at 9:11-19 (“As shown in FIGS. 1, 4A-4B, and 7A-

Claim	Claim Language	Infringement Evidence
		<p>7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heater-sensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the system 100, a set of elastic elements 160 configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 12:20-31 (“In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistance-to-voltage conversion circuit because the UT750 PID controller requires voltage as an input for PID control.”)U.S. Patent No. 9,499,896 at 11:63-12:4 “As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100.”
33(a)	A method of carrying out PCR on a plurality of samples, the method comprising:	<p>To the extent the preamble is limiting, the accused workflow is a method of carrying out PCR on a plurality of samples.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

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		 <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized

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		<p>reagents.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System.... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

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		<ul style="list-style-type: none"> <p>“This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59</p>  <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08</p> <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> <p>Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic</p>


Claim	Claim Language	Infringement Evidence
		<p>pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate.</p> <ul style="list-style-type: none"> Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste

Claim	Claim Language	Infringement Evidence
		<p>chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer.</p> <ul style="list-style-type: none"> • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the

Claim	Claim Language	Infringement Evidence
		<p>dichroic mirror, and the emission filter.</p> <ul style="list-style-type: none"> • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”) • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12

Claim	Claim Language	Infringement Evidence
		<p>different pathways for sample processing.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)
33(b)	introducing the plurality of samples into a multi-lane microfluidic cartridge, wherein each lane comprises a PCR reaction zone configured to permit thermal cycling of a sample independently of the other samples;	<p>The accused workflow includes introducing the plurality of samples into a multi-lane microfluidic cartridge, wherein each lane comprises a PCR reaction zone configured to permit thermal cycling of a sample independently of the other samples.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The liquid handling robot aspirates the PCR-ready solution and transfers it back to the cartridge where it dispenses into the same P-port from which the sample was aspirated.” <i>Id.</i> at 3:47-3:57

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Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 935 1108 967">US9101930 (Exhibit 25)</p> <ul style="list-style-type: none"> <li data-bbox="848 976 1923 1403"> <p>Claim 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising: a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer; a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, are each at least partially separated from the corrugated surface of the</p>

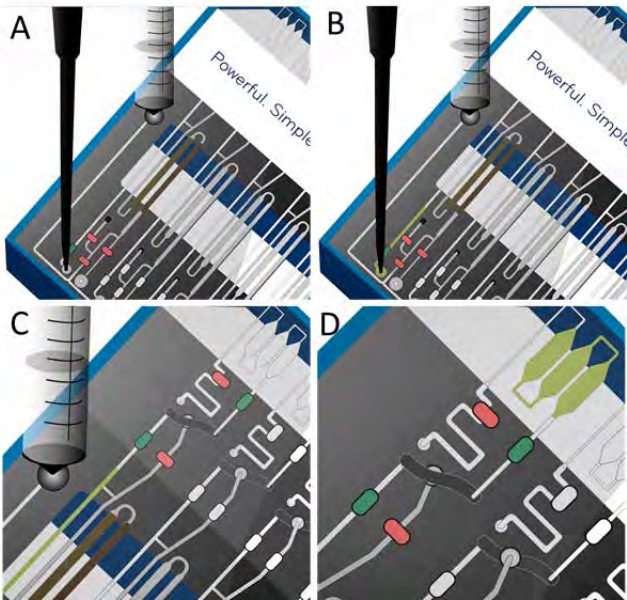
Claim	Claim Language	Infringement Evidence
		<p>intermediate substrate by an elastomeric layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate.</p> <ul style="list-style-type: none"> Claim 11. The cartridge of claim 10, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second

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		<p>sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward

Claim	Claim Language	Infringement Evidence
		<p>the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter.</p> <ul style="list-style-type: none"> • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) <p>U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to</p>

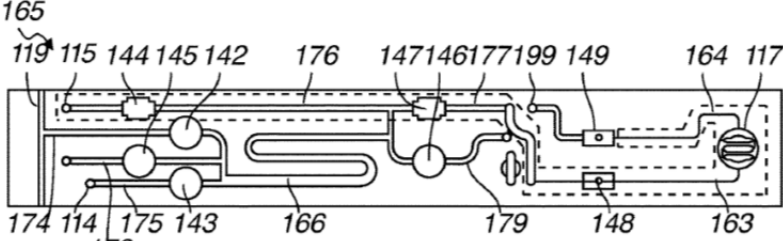
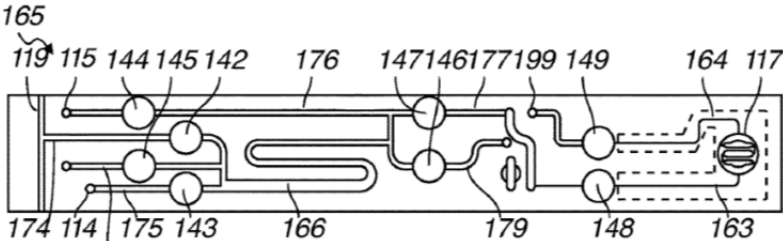
Claim	Claim Language	Infringement Evidence
		<p>the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers. U.S. Patent No. 9,499,896 at 12:15-20 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies 110.”) <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface


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		<p>with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,539,576 at 9:8-12 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies no.”) • U.S. Patent No. 9,539,576 at 12:59-64 (“Upon completion of Block S240, individual heater-sensor dies of the set of heater-sensor dies can be coupled to one or multiple electronics substrates in order to provide uniform heating of

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		individual sample containers with independent control of heating parameters provided at each of the set of heater-sensor dies.”)
33(c)	moving the plurality of samples into the respective plurality of PCR reaction zones; and	<p>The accused workflow includes moving the plurality of samples into the respective plurality of PCR reaction zones.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p>US9738887 (Exhibit 31)</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the

Claim	Claim Language	Infringement Evidence
		<p>detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • US Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • US Patent No. 9,738,887 at 15:31-35 (“The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.”) • US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth

Claim	Claim Language	Infringement Evidence
		<p>truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at Figs. 1J and 1K:  <p style="text-align: center;">FIG. 1J</p>  <p style="text-align: center;">FIG. 1K</p> <p style="text-align: right;">○ OCCLUDED</p>
33(d)	amplifying polynucleotides contained with the plurality of samples in the PCR reaction zones while thermal cycling the PCR reaction zones, at least one PCR reaction zone separately thermally controllable from another PCR reaction zone.	<p>The accused workflow includes amplifying polynucleotides contained with the plurality of samples in the PCR reaction zones while thermal cycling the PCR reaction zones, at least one PCR reaction zone separately thermally controllable from another PCR reaction zone.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers

Claim	Claim Language	Infringement Evidence
		<p>market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.”</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p><i>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</i></p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx

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		<p>Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.”</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

Claim	Claim Language	Infringement Evidence
		<div data-bbox="890 233 1793 776" data-label="Image"> </div> <ul style="list-style-type: none"> <li data-bbox="842 786 1923 1000"> <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p> <div data-bbox="905 1039 1612 1383" data-label="Image"> </div>

Claim	Claim Language	Infringement Evidence
		<p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module

Claim	Claim Language	Infringement Evidence
		<p>comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip,


Claim	Claim Language	Infringement Evidence
		<p>and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of

Claim	Claim Language	Infringement Evidence
		<p>nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) <p>U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate,

Claim	Claim Language	Infringement Evidence
		<p>comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,539,576 at 9:8-12 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies no.”) • U.S. Patent No. 9,539,576 at 12:59-64 (“Upon completion of Block S240, individual heater-sensor dies of the set of heater-sensor dies can be coupled to one or multiple electronics substrates in order to provide uniform heating of individual sample containers with independent control of heating parameters provided at each of the set of heater-sensor dies.”)


EXHIBIT 37

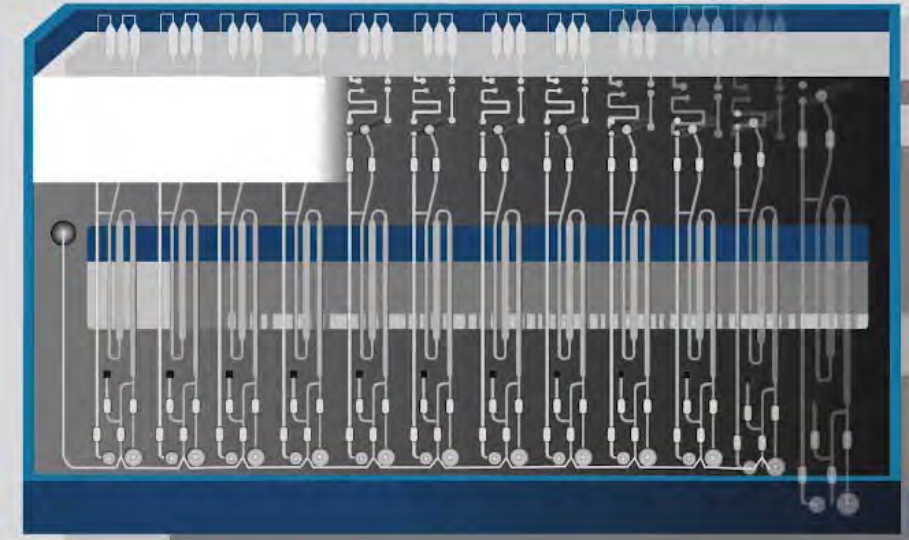
U.S. Patent No. 8,323,900 Infringement Chart


Claim	Claim Language	Infringement Evidence
1(a)	An apparatus, comprising:	<p>To the extent the preamble is limiting, the accused instrument is an apparatus.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p> <div data-bbox="793 513 1845 1325">  </div> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited</p>

Claim	Claim Language	Infringement Evidence
		<p>May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of

Claim	Claim Language	Infringement Evidence
		<p>the instrument with touchscreen computer, accessories, and reagents and consumables.”</p> <ul style="list-style-type: none"> • “NeuMoDx™ Molecular Systems are versatile; in addition to IVD tests, our system can also be used as an open system to process Laboratory Developed Tests (LDTs) that have been created and validated by your lab.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> • At 2:58-3:18 (“There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.”)
1(b)	a plurality of multi-lane microfluidic cartridges, each lane comprising a PCR reaction zone;	<p>The accused apparatus comprises a plurality of multi-lane microfluidic cartridges, each lane comprising a PCR reaction zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ TECHNOLOGY” hyperlink at https://player.vimeo.com/video/281470603. (Exhibit 17)</p> <ul style="list-style-type: none"> • at 4:55-5:00 (showing a plurality of multi-lane cartridges in the accused apparatus)

Claim	Claim Language	Infringement Evidence
		  <p data-bbox="793 1162 1900 1304"> <i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16) </p> <ul data-bbox="842 1312 1900 1414" style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

Claim	Claim Language	Infringement Evidence
		 <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

Claim	Claim Language	Infringement Evidence
		 <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized

Claim	Claim Language	Infringement Evidence
		<p>reagents.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System.... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p>“Patents”, http://www.neumodx.com/patents/, demonstrating that NeuMoDx marks its products with US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; 9,452,430; 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; 10,010,888; 9,382,532; 9,540,636; 9,499,896; 9,539,576; 9,637,775; and 10,093,963 (Exhibit 15)</p>

Claim	Claim Language	Infringement Evidence										
		<div><h2>PATENTS</h2><table><tr><th>Product</th><th>Patents</th></tr><tr><td>CARTRIDGE</td><td>US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.</td></tr><tr><td>P02 (overall system and method)</td><td>US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.</td></tr><tr><td>EXTRACTION PLATE</td><td>US Patent Nos. 9,382,532; and 9,540,636.</td></tr><tr><td>XPCR MODULE</td><td>US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.</td></tr></table></div> <div><p>US9403165 (Exhibit 27)</p><ul style="list-style-type: none">Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate.Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent</div>	Product	Patents	CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.	P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.	EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.	XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.
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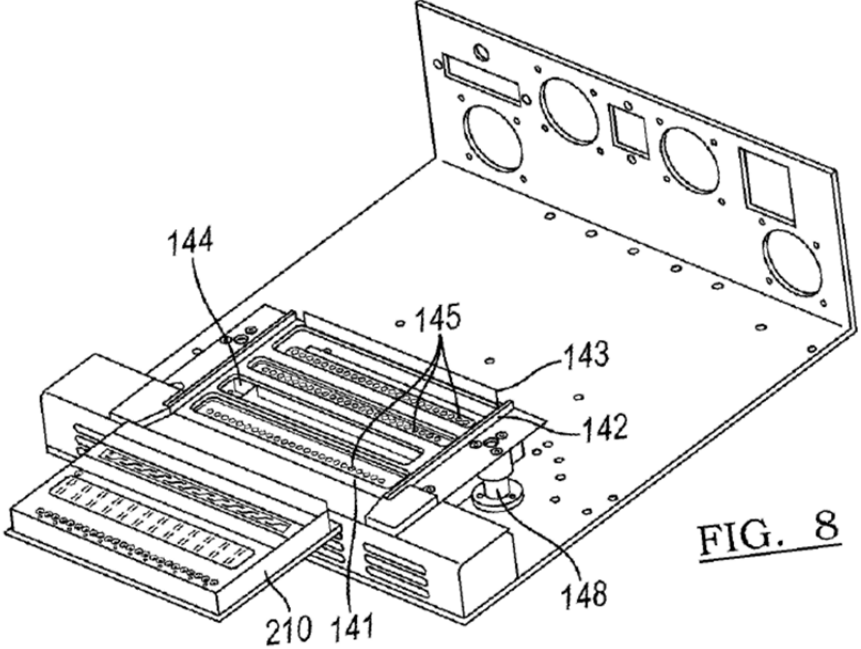
Claim	Claim Language	Infringement Evidence
		<p>port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular

Claim	Claim Language	Infringement Evidence
		<p>diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing

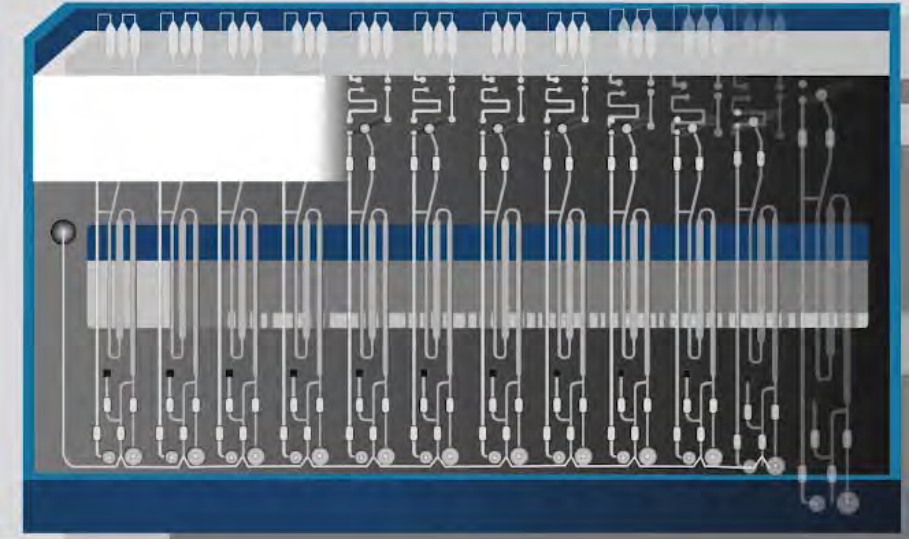
Claim	Claim Language	Infringement Evidence
		<p>and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis.

Claim	Claim Language	Infringement Evidence
		<p>Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)</p>
1(c)	a plurality of receiving bays, each receiving bay configured to receive one of the plurality of microfluidic cartridges;	<p>The accused apparatus comprises a plurality of receiving bays, each receiving bay configured to receive one of the plurality of microfluidic cartridges.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ TECHNOLOGY” hyperlink at https://player.vimeo.com/video/281470603. (Exhibit 17)</p> <ul style="list-style-type: none"> • at 4:55-5:00

Claim	Claim Language	Infringement Evidence
		  <p data-bbox="793 1122 1113 1159">US9050594 (Exhibit 24)</p> <ul data-bbox="840 1166 1921 1414" style="list-style-type: none"> • Claim 1. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator

Claim	Claim Language	Infringement Evidence
		<p>configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 2:6-7 (“FIG. 8 depicts an embodiment of a microfluidic cartridge and an embodiment of a cartridge platform.”) • U.S. Patent No. 9,050,594 at Fig. 8  <p style="text-align: right;">FIG. 8</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 7:53-8:35 “As shown in FIG. 9A, the cartridge receiving module 140 of the molecular diagnostic module 130 comprises a cartridge platform 141 including a cartridge loading guiderail 142, a cartridge

Claim	Claim Language	Infringement Evidence
		<p>stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145; a linear actuator 146 configured to displace a microfluidic cartridge 210 resting on the cartridge platform 141, and a set of springs 148 coupled to the cartridge platform 141. The cartridge receiving module 140 thus functions to receive, align, and compress a microfluidic cartridge 210 for processing of a biological sample according to a molecular diagnostic assay protocol.... The cartridge platform 141 includes a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145, and functions to receive and align a microfluidic cartridge 210, while providing access to the microfluidic cartridge 210 by a magnet 160 and a valve actuation subsystem 170. As shown in FIG. 8, an embodiment of the cartridge platform 141 includes a pair of parallel cartridge loading guiderails 142, initiating at a pair of inwardly tapering protrusions configured to guide a microfluidic cartridge toward the pair of parallel cartridge loading guiderails 142, and spanning two short edges of the cartridge platform 141. The embodiment of the cartridge platform 141 also includes a cartridge stop 143 comprising a vertical tab oriented perpendicular to the cartridge loading guiderails 142, and spanning a long edge of the cartridge platform. Preferably, the cartridge loading guiderails 142 and the cartridge stop 143 are configured such that a microfluidic cartridge 210 slides between the cartridge loading guiderails 142 and hits the cartridge stop 143 to signal proper alignment.”</p>
1(d)	each PCR reaction zone comprising a separately controllable heat source thermally coupled thereto,	<p>In the accused apparatus, each PCR reaction zone comprises a separately controllable heat source thermally coupled thereto.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

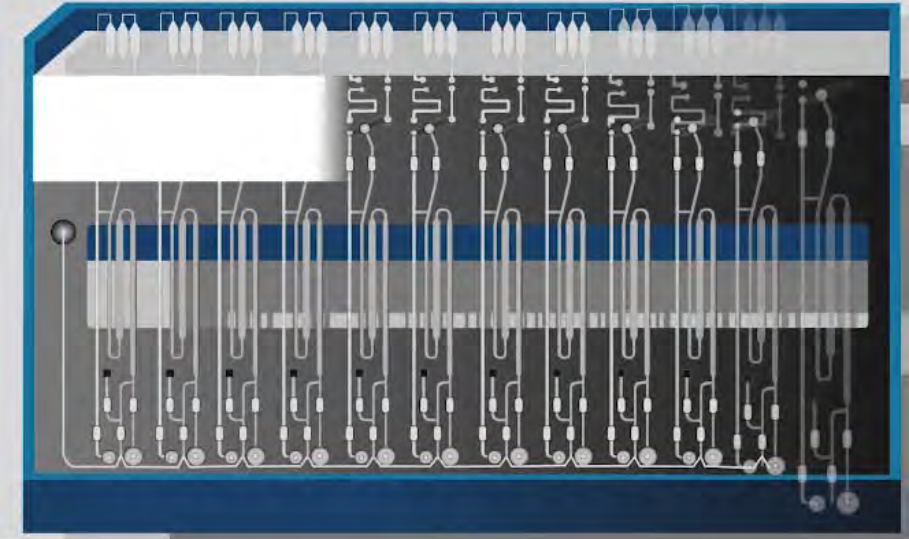
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 813 1877 889"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="842 894 1921 1219" style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p data-bbox="793 1260 1108 1292">US9050594 (Exhibit 24)</p> <ul data-bbox="842 1300 1921 1403" style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic

Claim	Claim Language	Infringement Evidence
		<p>bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-

Claim	Claim Language	Infringement Evidence
		<p>sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12

Claim	Claim Language	Infringement Evidence
		<p>different pathways for sample processing.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”) <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of

Claim	Claim Language	Infringement Evidence
		<p>the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <p>U.S. Patent No. 9,499,896 at 12:15-20 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies 110.”)</p>
1(e)	<p>wherein the heat source thermal cycles the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone and</p>	<p>In the accused apparatus, each PCR reaction zone comprises a separately controllable heat source thermally coupled thereto wherein the heat source thermal cycles the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

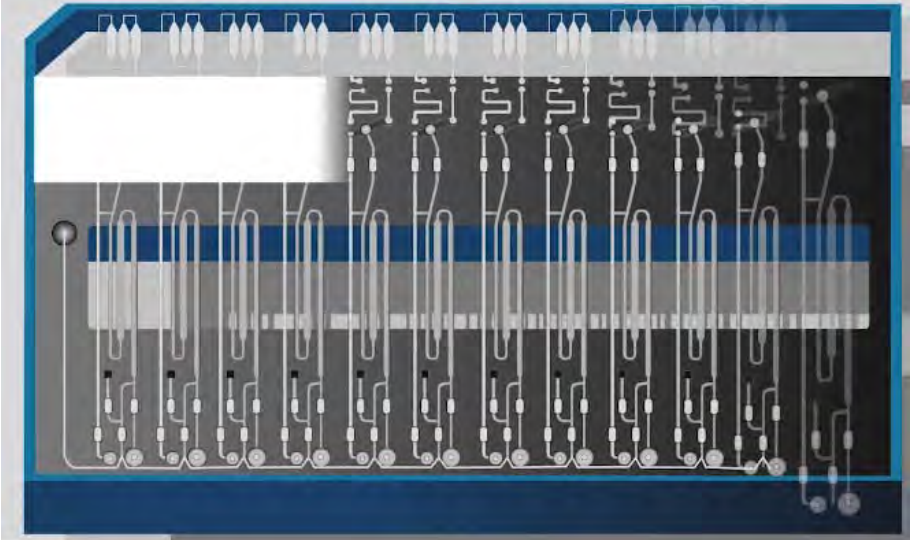
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 816 1877 889"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="846 898 1921 1222" style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p data-bbox="793 1263 1108 1295">US9050594 (Exhibit 24)</p> <ul data-bbox="846 1304 1921 1403" style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic

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		<p>bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-

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		<p>sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12

Claim	Claim Language	Infringement Evidence
		<p>different pathways for sample processing.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”) <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of

Claim	Claim Language	Infringement Evidence
		the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.
1(f)	maintains a substantially uniform temperature throughout the PCR reaction zone during each cycle;	<p>In the accused apparatus, each PCR reaction zone comprises a separately controllable heat source thermally coupled thereto wherein the heat source thermal cycles the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone and maintains a substantially uniform temperature throughout the PCR reaction zone during each cycle.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

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		 <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

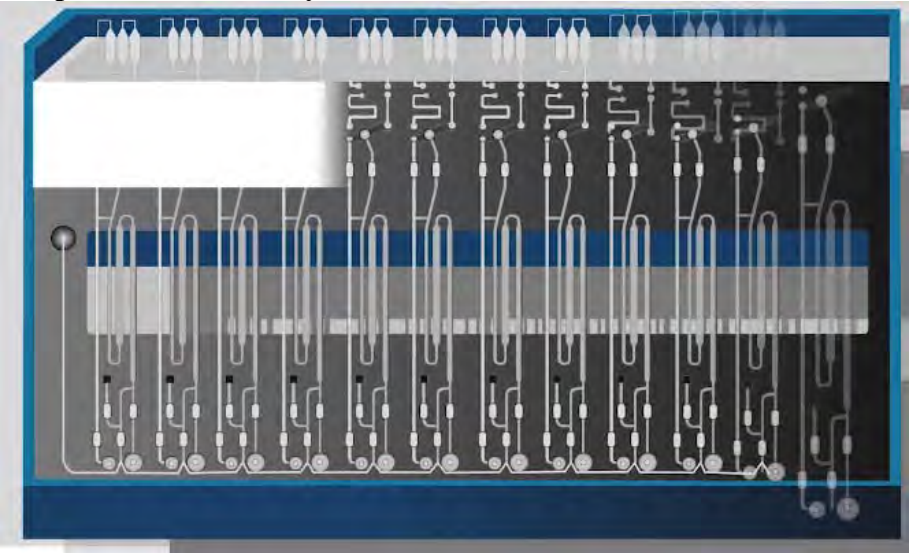
Claim	Claim Language	Infringement Evidence
		<div data-bbox="905 228 1612 574" data-label="Image"> </div> <p data-bbox="793 613 1877 683"><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="842 695 1921 1019" style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p data-bbox="793 1057 1108 1089">US9050594 (Exhibit 24)</p> <ul data-bbox="842 1101 1921 1421" style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet

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		<p>receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module

Claim	Claim Language	Infringement Evidence
		<p>comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter.</p> <ul style="list-style-type: none"> • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic

Claim	Claim Language	Infringement Evidence
		<p>pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers. U.S. Patent No. 9,499,896 at 2:33-48 “The system 100 functions to enable

Claim	Claim Language	Infringement Evidence
		<p>rapid thermal cycling of samples while providing uniform heating and preventing signal drift. In specific applications, the system 100 can be used to rapidly and controllably thermocycle nucleic acid samples during performance of molecular diagnostic amplification techniques (e.g., PCR, RT-PCR), signal amplification techniques (e.g., bDNA, hybrid capture), and analytical techniques (e.g., gel electrophoresis, mass spectrometry). The system 100 can also provide rapid thermocycling without significant power requirements, ensure a closer correlation between the actual heating temperature and the temperature set-point by implementing an integrated heater-sensor die, and controllably and individually heat small sample volumes (e.g., picoliters, nanoliters) based upon a microfabrication technique that also enables mass production of the system 100.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:61-3:3 (“The set of heater-sensor dies 110 functions to controllably heat individual sample volumes. Preferably, each heater sensor die 111 is a thin-film die that can be deposited onto another substrate (e.g., silicon, glass substrate) that can be packaged onto an electronics substrate 140 (e.g., printed circuit board, PCB); however, each heater-sensor die 111 can alternatively comprise any suitable geometry and/or configuration that enables controlled, uniform, and rapid heating of a detection chamber in thermal communication with the heater-sensor die 111.”) • U.S. Patent No. 9,499,896 at 3:23-27 (“Preferably, each heater-sensor die 111 in the set of heater sensor dies 110 comprises an assembly including: a first insulating layer 112a that functions to provide an insulating barrier to isolate the heaters and sensors and a heating region 113 that functions to provide uniform sample heating.”)
1(g)	a detector configured to detect the presence of an amplification product in one or more PCR reaction zones; and	<p>The accused apparatus comprises a detector configured to detect the presence of an amplification product in one or more PCR reaction zones.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO </p>

Claim	Claim Language	Infringement Evidence
		<p>NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the

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		<p>heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:33-48 “The system 100 functions to enable rapid thermal cycling of samples while providing uniform heating and preventing signal drift. In specific applications, the system 100 can be used to rapidly and controllably thermocycle nucleic acid samples during performance of molecular diagnostic amplification techniques (e.g., PCR, RT-PCR), signal amplification techniques (e.g., bDNA, hybrid capture), and analytical techniques (e.g., gel electrophoresis, mass spectrometry). The system 100 can also provide rapid thermocycling without significant power requirements, ensure a closer correlation between the actual heating temperature and the temperature set-point by implementing an integrated heater-sensor die, and controllably and individually heat small sample volumes (e.g., picoliters, nanoliters) based upon a microfabrication technique that also enables mass production of the system 100.”) • U.S. Patent No. 9,499,896 at 2:61-3:3 (“The set of heater-sensor dies 110 functions to controllably heat individual sample volumes. Preferably, each heater sensor die 111 is a thin-film die that can be deposited onto another substrate (e.g., silicon, glass substrate) that can be packaged onto an electronics substrate 140 (e.g., printed circuit board, PCB); however, each heater-sensor die 111 can alternatively comprise any suitable geometry and/or configuration that enables controlled, uniform, and rapid heating of a detection chamber in thermal communication with the heater-sensor die


Claim	Claim Language	Infringement Evidence
		<p>111.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 3:23-27 (“Preferably, each heater-sensor die 111 in the set of heater sensor dies 110 comprises an assembly including: a first insulating layer 112a that functions to provide an insulating barrier to isolate the heaters and sensors and a heating region 113 that functions to provide uniform sample heating.”) <p style="text-align: center;">FIG. 12A</p>
1(h)	a processor coupled to the detector and the heat sources, configured to control heating of one or more PCR reaction zones by the heat sources.	<p>The accused apparatus comprises a processor coupled to the detector and the heat sources, configured to control heating of one or more PCR reaction zones by the heat sources.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully

Claim	Claim Language	Infringement Evidence
		<p>automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/dr-steven-</p>


Claim	Claim Language	Infringement Evidence
		<p>young-video-testimonial/, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> At 2:58-3:18 (“There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.”) <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of

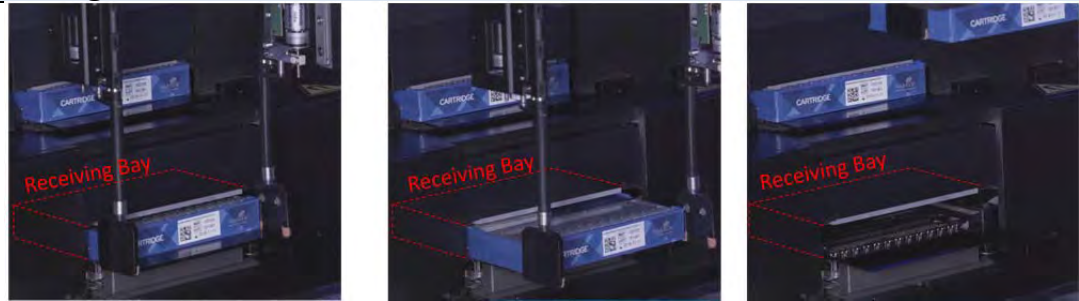
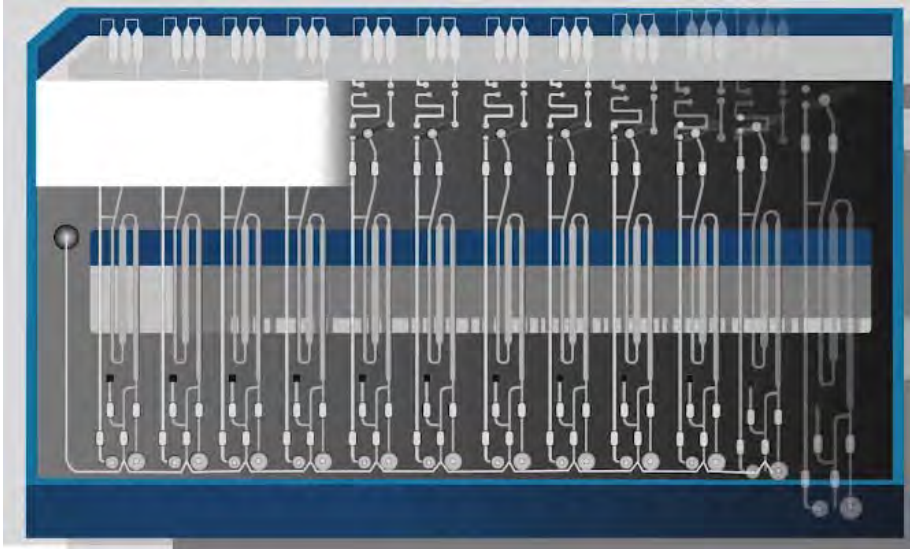
Claim	Claim Language	Infringement Evidence
		<p>detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers. U.S. Patent No. 9,499,896 at 2:21-32 (“As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the


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		<p>electronics substrate and configured to bias each of the set of heater-sensor dies against a detection 30 chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 9:11-19 (“As shown in FIGS. 1, 4A-4B, and 7A-7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heater-sensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the system 100, a set of elastic elements 160 configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.”) • U.S. Patent No. 9,499,896 at 12:20-31 (“In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistance-to-voltage conversion circuit because the UT750 PID controller requires voltage as an input for PID control.”) • U.S. Patent No. 9,499,896 at 11:63-12:4 “As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100.”)
7(a)	A device for carrying out PCR on a plurality of samples, the	To the extent the preamble is limiting, the accused instrument is a device.

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	device comprising:	<p data-bbox="793 233 1913 302"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p> <div data-bbox="793 302 1845 1114">  </div> <p data-bbox="793 1149 1913 1224"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="842 1230 1892 1305" style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result.” <p data-bbox="793 1338 1913 1408"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “NeuMoDx™ Molecular Systems are versatile; in addition to IVD tests, our system can also be used as an open system to process Laboratory Developed Tests (LDTs) that have been created and validated by your lab.”

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		<p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> At 2:58-3:18 (“There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.”)
7(b)	a plurality of multi-lane microfluidic cartridges, each lane comprising a PCR reaction zone;	<p>The accused device comprises a plurality of multi-lane microfluidic cartridges, each lane comprising a PCR reaction zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ TECHNOLOGY” hyperlink at https://player.vimeo.com/video/281470603. (Exhibit 17)</p> <ul style="list-style-type: none"> at 4:55-5:00 (showing a plurality of multi-lane cartridges in the accused apparatus) 

Claim	Claim Language	Infringement Evidence
		<div data-bbox="793 228 1864 527">  </div> <p data-bbox="793 602 1900 743"> <i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16) </p> <ul data-bbox="842 751 1892 857" style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59 <div data-bbox="890 857 1793 1403">  </div>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”.


Claim	Claim Language	Infringement Evidence
		<p>The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System.... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx


Claim	Claim Language	Infringement Evidence
		<p>System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.”</p> <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic

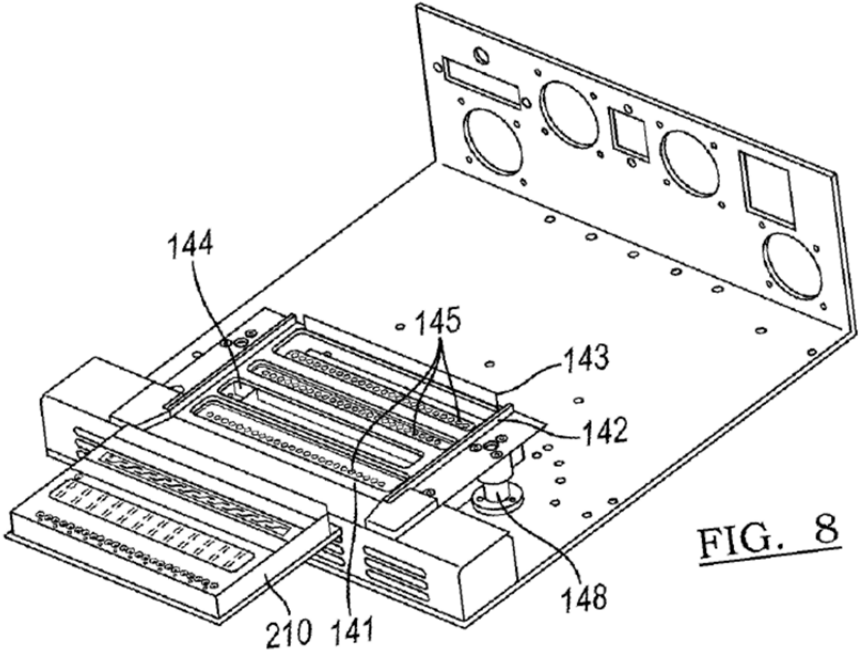
Claim	Claim Language	Infringement Evidence
		<p>bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-

Claim	Claim Language	Infringement Evidence
		<p>sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system

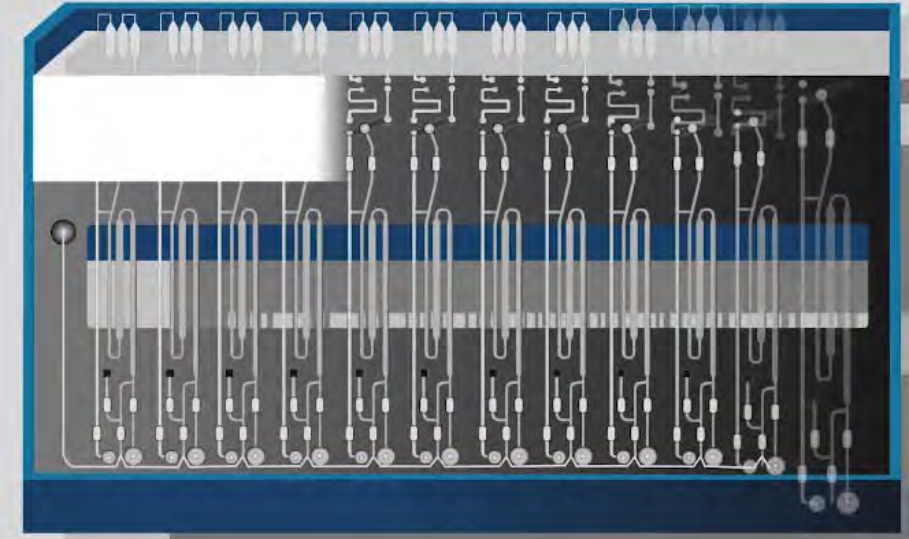
Claim	Claim Language	Infringement Evidence
		<p>configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)
7(c)	a plurality of receiving bays, each receiving bay configured to	The accused device comprises a plurality of receiving bays, each receiving bay configured to receive one of the plurality of microfluidic cartridges.

Claim	Claim Language	Infringement Evidence
	receive one of the plurality of microfluidic cartridges;	<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ TECHNOLOGY” hyperlink at https://player.vimeo.com/video/281470603. (Exhibit 17)</p> <ul style="list-style-type: none"> • at 4:55-5:00 

Claim	Claim Language	Infringement Evidence
		<div data-bbox="798 228 1864 527">  </div> <p data-bbox="798 565 1113 597">US9050594 (Exhibit 24)</p> <ul data-bbox="846 605 1919 1261" style="list-style-type: none"> <li data-bbox="846 605 1919 1117">• Claim 1. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. <li data-bbox="846 1157 1808 1222">• U.S. Patent No. 9,050,594 at 2:6-7 (“FIG. 8 depicts an embodiment of a microfluidic cartridge and an embodiment of a cartridge platform.”) <li data-bbox="846 1230 1339 1261">• U.S. Patent No. 9,050,594 at Fig. 8

Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1648 738 1795 803">FIG. 8</p> <ul style="list-style-type: none"> U.S. Patent No. 9,050,594 at 7:53-8:35 “As shown in FIG. 9A, the cartridge receiving module 140 of the molecular diagnostic module 130 comprises a cartridge receiving module 140 including a cartridge platform 141 including a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145; a linear actuator 146 configured to displace a microfluidic cartridge 210 resting on the cartridge platform 141, and a set of springs 148 coupled to the cartridge platform 141. The cartridge receiving module 140 thus functions to receive, align, and compress a microfluidic cartridge 210 for processing of a biological sample according to a molecular diagnostic assay protocol.... The cartridge platform 141 includes a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145, and functions to receive and align a microfluidic cartridge 210, while providing access to the microfluidic cartridge 210 by a magnet 160 and a valve actuation subsystem 170. As shown in FIG. 8, an embodiment of the cartridge

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		<p>platform 141 includes a pair of parallel cartridge loading guiderails 142, initiating at a pair of inwardly tapering protrusions configured to guide a microfluidic cartridge toward the pair of parallel cartridge loading guiderails 142, and spanning two short edges of the cartridge platform 141. The embodiment of the cartridge platform 141 also includes a cartridge stop 143 comprising a vertical tab oriented perpendicular to the cartridge loading guiderails 142, and spanning a long edge of the cartridge platform. Preferably, the cartridge loading guiderails 142 and the cartridge stop 143 are configured such that a microfluidic cartridge 210 slides between the cartridge loading guiderails 142 and hits the cartridge stop 143 to signal proper alignment.”</p>
7(d)	a separately controllable heat source thermally coupled to each PCR reaction zone,	<p>The accused device comprises a separately controllable heat source thermally coupled to each PCR reaction zone.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

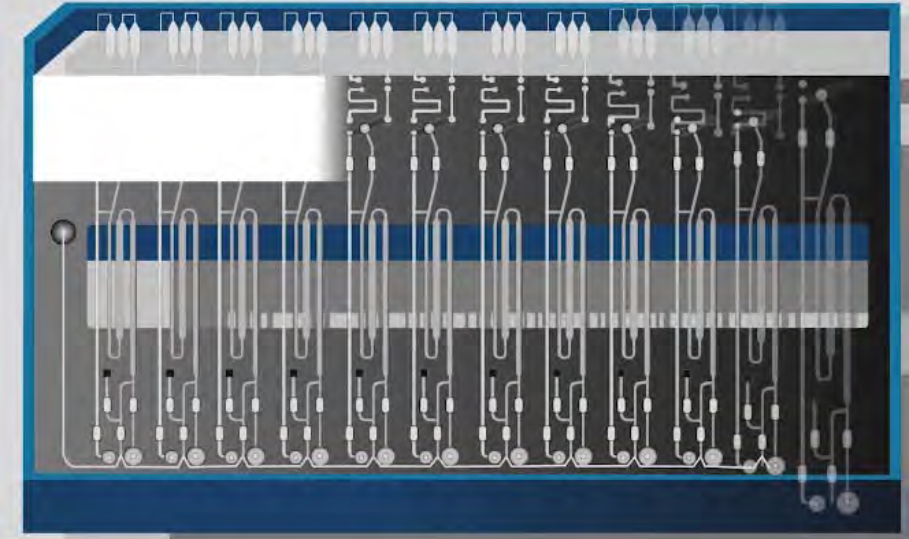
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 816 1877 889"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="846 898 1921 1222" style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p data-bbox="793 1263 1108 1295">US9050594 (Exhibit 24)</p> <ul data-bbox="846 1304 1921 1403" style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic

Claim	Claim Language	Infringement Evidence
		<p>bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-

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		<p>sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12

Claim	Claim Language	Infringement Evidence
		<p>different pathways for sample processing.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”) <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of

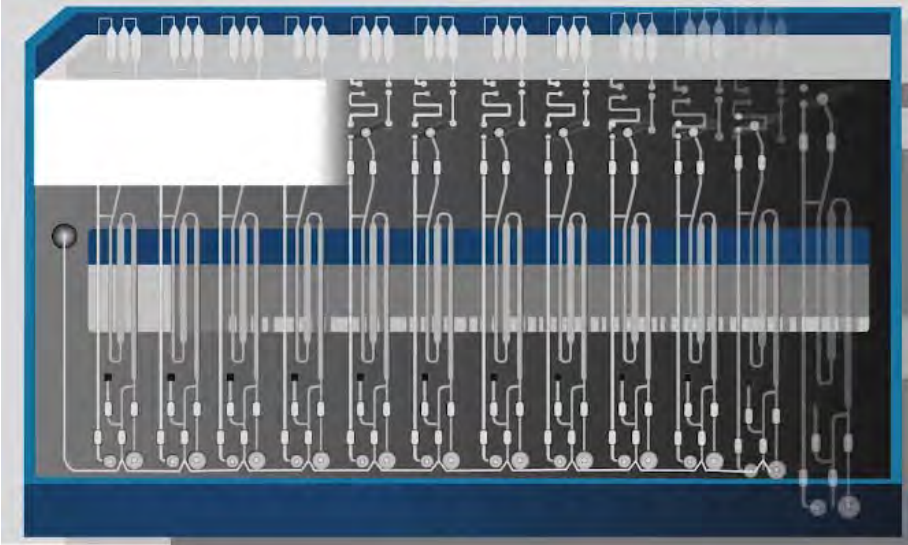
Claim	Claim Language	Infringement Evidence
		<p>the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <p>U.S. Patent No. 9,499,896 at 12:15-20 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies 110.”)</p>
7(e)	wherein the heat source is configured to thermal cycle the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone and	<p>In the accused device, the heat source is configured to thermal cycle the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

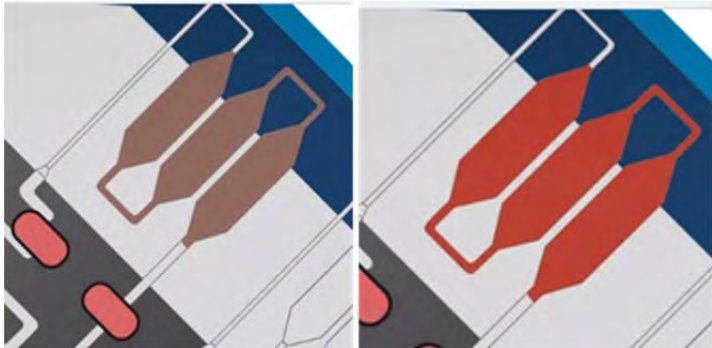
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		 <p data-bbox="793 816 1877 889"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="846 898 1921 1222" style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p data-bbox="793 1263 1108 1295">US9050594 (Exhibit 24)</p> <ul data-bbox="846 1304 1921 1403" style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic

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		<p>bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-

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		<p>sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12

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		<p>different pathways for sample processing.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”) <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of

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		<p>the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p>
7(f)	<p>to maintain a substantially uniform temperature throughout the PCR reaction zone during each cycle;</p>	<p>In the accused device, the heat source is configured to thermal cycle the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone and to maintain a substantially uniform temperature throughout the PCR reaction zone during each cycle</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59 

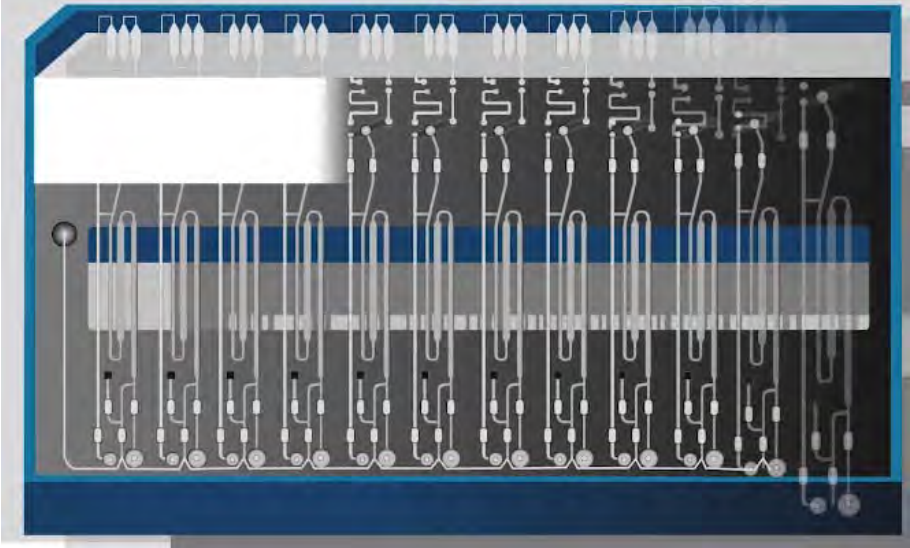
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p>  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> <p>“NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.”</p>

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		<p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic

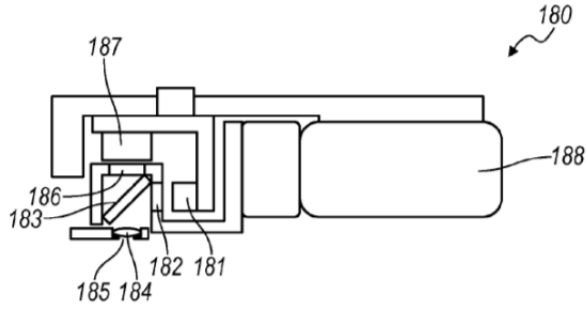
Claim	Claim Language	Infringement Evidence
		<p>module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157,

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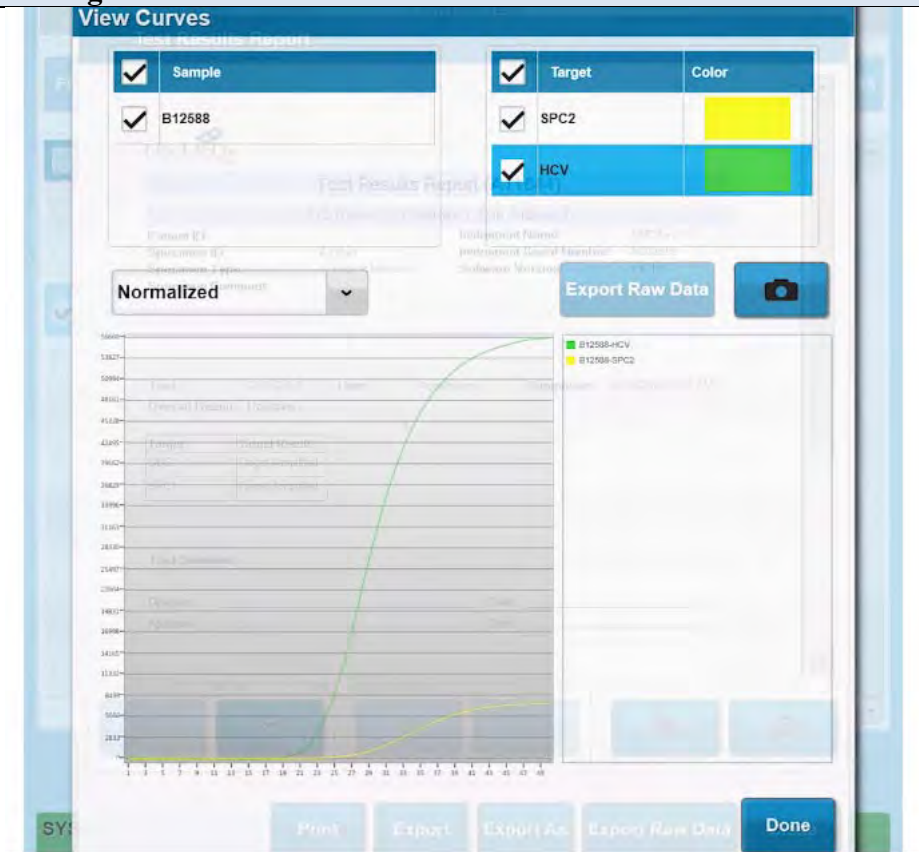
Claim	Claim Language	Infringement Evidence
		<p>associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 2:33-48 “The system 100 functions to enable rapid thermal cycling of samples while providing uniform heating and preventing signal drift. In specific applications, the system 100 can be used to rapidly and controllably thermocycle nucleic acid samples during performance of molecular diagnostic amplification techniques (e.g., PCR, RT-PCR), signal amplification techniques (e.g., bDNA, hybrid capture), and analytical techniques (e.g., gel electrophoresis, mass spectrometry). The system 100 can also provide rapid thermocycling without significant power requirements, ensure a closer correlation between the actual heating temperature and the temperature set-point by implementing an integrated heater-sensor die, and controllably and individually heat small sample volumes (e.g., picoliters, nanoliters) based upon a microfabrication technique that also enables mass production of the system 100.”) U.S. Patent No. 9,499,896 at 2:61-3:3 (“The set of heater-sensor dies 110 functions to controllably heat individual sample volumes. Preferably, each heater sensor die 111 is a thin-film die that can be deposited onto another substrate (e.g., silicon, glass substrate) that can be packaged onto an electronics substrate 140 (e.g., printed circuit board, PCB); however, each heater-sensor die 111 can alternatively comprise any suitable geometry and/or configuration that enables controlled, uniform, and rapid heating of a detection chamber in thermal communication with the heater-sensor die 111.”) U.S. Patent No. 9,499,896 at 3:23-27 (“Preferably, each heater-sensor die 111 in


Claim	Claim Language	Infringement Evidence
		<p>the set of heater sensor dies 110 comprises an assembly including: a first insulating layer 112a that functions to provide an insulating barrier to isolate the heaters and sensors and a heating region 113 that functions to provide uniform sample heating.")</p>
7(g)	<p>a detector configured to detect the presence of an amplification product in one or more PCR reaction zones;</p>	<p>The accused device comprises a detector configured to detect the presence of an amplification product in one or more PCR reaction zones.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to "VIDEO NeuMoDx™ WORKFLOW" hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • "This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously." <i>Id.</i> at 1:49-1:59 

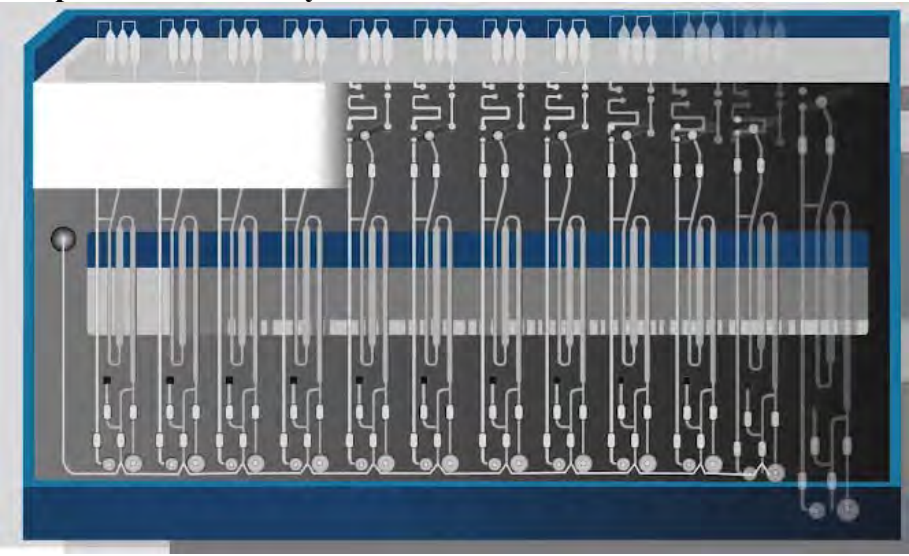
Claim	Claim Language	Infringement Evidence
		<p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers. U.S. Patent No. 9,499,896 at 2:33-48 “The system 100 functions to enable rapid thermal cycling of samples while providing uniform heating and preventing signal drift. In specific applications, the system 100 can be used to rapidly and controllably thermocycle nucleic acid samples during performance of molecular diagnostic amplification techniques (e.g., PCR, RT-PCR), signal amplification techniques (e.g., bDNA, hybrid capture), and analytical techniques (e.g., gel electrophoresis, mass spectrometry). The system 100 can also provide rapid thermocycling without significant power requirements, ensure a closer correlation between the actual heating temperature and the temperature set-point by implementing an integrated heater-sensor die, and controllably and individually heat small sample volumes (e.g., picoliters, nanoliters) based upon

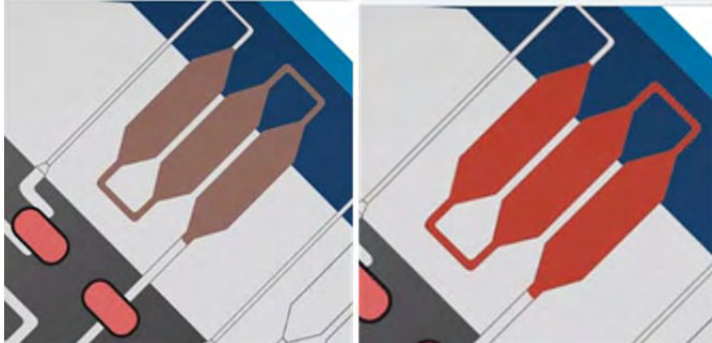
Claim	Claim Language	Infringement Evidence
		<p>a microfabrication technique that also enables mass production of the system 100.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 2:61-3:3 (“The set of heater-sensor dies 110 functions to controllably heat individual sample volumes. Preferably, each heater sensor die 111 is a thin-film die that can be deposited onto another substrate (e.g., silicon, glass substrate) that can be packaged onto an electronics substrate 140 (e.g., printed circuit board, PCB); however, each heater-sensor die 111 can alternatively comprise any suitable geometry and/or configuration that enables controlled, uniform, and rapid heating of a detection chamber in thermal communication with the heater-sensor die 111.”) U.S. Patent No. 9,499,896 at 3:23-27 (“Preferably, each heater-sensor die 111 in the set of heater sensor dies 110 comprises an assembly including: a first insulating layer 112a that functions to provide an insulating barrier to isolate the heaters and sensors and a heating region 113 that functions to provide uniform sample heating.”)  <p style="text-align: center;">FIG. 12A</p>
7(h)	a processor coupled to the detector	<p>The accused device comprises a processor coupled to the detector.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited</p>


Claim	Claim Language	Infringement Evidence
		<p>May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

Claim	Claim Language	Infringement Evidence
		 <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> Describing "...microfluidic cartridges capable of performing independent sample processing and real-time PCR."

Claim	Claim Language	Infringement Evidence
		 <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access

Claim	Claim Language	Infringement Evidence
		<p>processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

Claim	Claim Language	Infringement Evidence
		 <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.”
7(i)	and a plurality of the separately controllable heat sources, configured to control heating of one or more PCR reaction zones by one or more of the plurality of separately controllable heat sources; and	<p>The accused device comprises a plurality of the separately controllable heat sources, configured to control heating of one or more PCR reaction zones by one or more of the plurality of separately controllable heat sources.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

Claim	Claim Language	Infringement Evidence
		 <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

Claim	Claim Language	Infringement Evidence
		<div data-bbox="890 233 1793 776" data-label="Image"> </div> <ul style="list-style-type: none"> <li data-bbox="842 786 1923 1000"> <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p> <div data-bbox="905 1039 1612 1383" data-label="Image"> </div>

Claim	Claim Language	Infringement Evidence
		<p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic


Claim	Claim Language	Infringement Evidence
		<p>acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads,

Claim	Claim Language	Infringement Evidence
		<p>isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently

Claim	Claim Language	Infringement Evidence
		<p>of the other nucleic acid reagent mixtures.”)</p> <ul style="list-style-type: none"> •
7(j)	an input device coupled to the processor and configured to permit concurrent or consecutive control of the plurality of multi-lane microfluidic cartridges	<p>The accused device comprises an input device coupled to the processor and configured to permit concurrent or consecutive control of the plurality of multi-lane microfluidic cartridges.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieveing [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated

Claim	Claim Language	Infringement Evidence						
		<p>extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.”</p> <ul style="list-style-type: none">• “NeuMoDx™ Molecular Systems are versatile; in addition to IVD tests, our system can also be used as an open system to process Laboratory Developed Tests (LDTs) that have been created and validated by your lab.” <p>JFO_2018-10-25_8009-Rev-B_NeuMoDx-96-Spec-Sheet.pdf (Exhibit 21)</p> <table><tr><td>Sample capacity</td><td>96 initial load; Continuous, Random-Access Thereafter</td></tr><tr><td>Reagent capacity</td><td>320 initial load; Continuous, Random-Access Thereafter</td></tr></table> <table><tr><td>Operational flexibility</td><td>Continuous Random-Access Perform LDT Qualitative and Quantitative assays simultaneously on demand⁴ Onboard inventory management Simultaneous use of multiple tube types and sizes Flexible specimen tube compatibility<ul style="list-style-type: none">• Diameter: 11 mm - 18 mm• Height: 60 mm – 120 mm</td></tr></table> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX , http://www.neumodx.com/product/neumodx-288/, last visited June 4, 2019 (Exhibit 13)</p> <ul style="list-style-type: none">• “The NeuMoDx™ 288 Molecular System is intended for in vitro diagnostic (IVD) use in performing NeuMoDx™ validated nucleic acid testing in clinical laboratories. The NeuMoDx™ 288 Molecular System is capable of automated extraction and isolation of nucleic acids from multiple specimen types, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The system is capable of providing functionality to enable laboratories to develop qualitative and quantitative tests, which use NeuMoDx™ -provided consumables and reagents.• Instrument Includes:<ul style="list-style-type: none">○ Uninterruptible power supply (UPS)	Sample capacity	96 initial load; Continuous, Random-Access Thereafter	Reagent capacity	320 initial load; Continuous, Random-Access Thereafter	Operational flexibility	Continuous Random-Access Perform LDT Qualitative and Quantitative assays simultaneously on demand ⁴ Onboard inventory management Simultaneous use of multiple tube types and sizes Flexible specimen tube compatibility <ul style="list-style-type: none">• Diameter: 11 mm - 18 mm• Height: 60 mm – 120 mm
Sample capacity	96 initial load; Continuous, Random-Access Thereafter							
Reagent capacity	320 initial load; Continuous, Random-Access Thereafter							
Operational flexibility	Continuous Random-Access Perform LDT Qualitative and Quantitative assays simultaneously on demand ⁴ Onboard inventory management Simultaneous use of multiple tube types and sizes Flexible specimen tube compatibility <ul style="list-style-type: none">• Diameter: 11 mm - 18 mm• Height: 60 mm – 120 mm							


Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> ○ Handheld barcode scanner ○ Keyboard and mouse ○ NeuMoDx™ Biohazard Waste Container ○ Carriers ○ Test Strip Carrier (6) ○ Buffer Carrier (2) ○ 32-tube Specimen Tube Carrier (9) ○ Tip, Extraction and Filter Carrier (2) ○ Cartridge Carrier (2)” <p><i>NeuMoDx™ Molecular Systems, NEUMODX ,</i> http://www.neumodx.com/product/neumodx-96/, last visited June 4, 2019 (Exhibit 14)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ 96 Molecular System is intended for in vitro diagnostic (IVD) use in performing NeuMoDx™ validated nucleic acid testing in clinical laboratories. The NeuMoDx™ 96 Molecular System is capable of automated extraction and isolation of nucleic acids from multiple specimen types, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The system is capable of providing functionality to enable laboratories to develop qualitative and quantitative tests, which use NeuMoDx™ provided consumables and reagents. • Instrument Includes: <ul style="list-style-type: none"> ○ Uninterruptible power supply (UPS) ○ Handheld barcode scanner ○ Keyboard and mouse ○ Biohazard Waste Bin ○ Biohazard Tip Waste Bin ○ Biohazard Waste Container ○ Carriers ○ Test Strip Carrier (4) ○ Buffer Carrier (1) ○ 32-tube Specimen Tube Carrier (3) ○ Tip, Extraction and Filter Carrier (1)

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> Cartridge Carrier (1)”
20(a)	A method of carrying out PCR on a plurality of samples, the method comprising:	<p>To the extent the preamble is limiting, the accused workflow is a method of carrying out PCR on a plurality of samples.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p>  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited</p>

Claim	Claim Language	Infringement Evidence
		<p>May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of

Claim	Claim Language	Infringement Evidence
		<p>the instrument with touchscreen computer, accessories, and reagents and consumables.”</p> <ul style="list-style-type: none"> • “NeuMoDx™ Molecular Systems are versatile; in addition to IVD tests, our system can also be used as an open system to process Laboratory Developed Tests (LDTs) that have been created and validated by your lab.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/dr-steven-young-video-testimonial/, hyperlink at https://youtu.be/vukP6gbLBYE. (Exhibit 32)</p> <ul style="list-style-type: none"> • At 2:58-3:18 (“There’s two systems that have been put into operation by NeuMoDx. One is the 288. It’s a high-throughput instrument, versus the 96, which is a lower throughput instrument. The advantage is that both systems use all of the same chemistry and all of the same hardware. Its just a smaller footprint.”)
20(b)	introducing the plurality of samples into a plurality of multi-lane microfluidic cartridges, wherein each lane comprises a PCR reaction zone configured to permit thermal cycling of a sample independently of the other samples;	<p>The accused workflow comprises introducing the plurality of samples into a plurality of multi-lane microfluidic cartridges, wherein each lane comprises a PCR reaction zone configured to permit thermal cycling of a sample independently of the other samples</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The liquid handling robot aspirates the PCR-ready solution and transfers it back to the cartridge where it dispenses into the same P-port from which the sample was aspirated.” <i>Id.</i> at 3:47-3:57

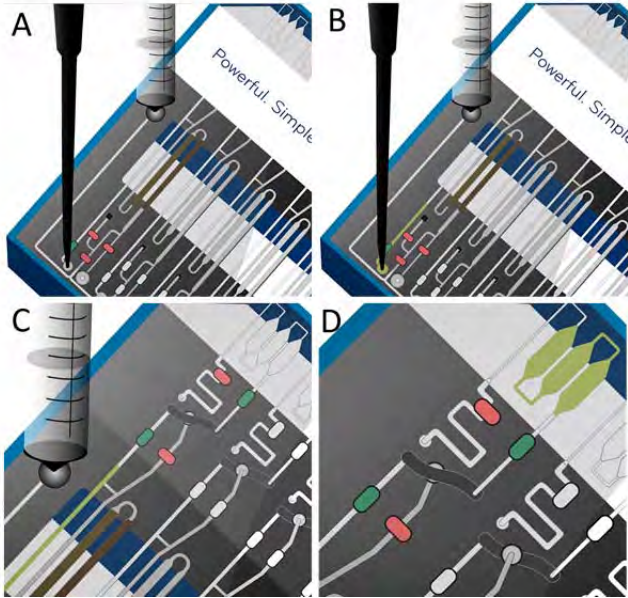
Claim	Claim Language	Infringement Evidence
		

Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 935 1108 967">US9101930 (Exhibit 25)</p> <ul data-bbox="848 976 1923 1403" style="list-style-type: none"> • Claim 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising: a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer; a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, are each at least partially separated from the corrugated surface of the

Claim	Claim Language	Infringement Evidence
		<p>intermediate substrate by an elastomeric layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate.</p> <ul style="list-style-type: none"> Claim 11. The cartridge of claim 10, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second

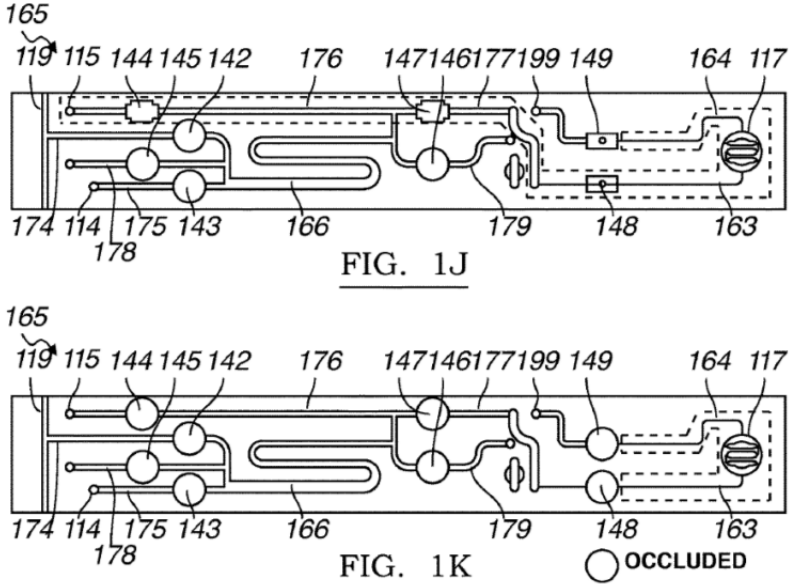
Claim	Claim Language	Infringement Evidence
		<p>sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward


Claim	Claim Language	Infringement Evidence
		<p>the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter.</p> <ul style="list-style-type: none"> • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but

Claim	Claim Language	Infringement Evidence
		<p>alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)</p>
20(c)	<p>moving the plurality of samples into the respective plurality of PCR reaction zones; and</p>	<p>The accused workflow comprises moving the plurality of samples into the respective plurality of PCR reaction zones.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 

Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion

Claim	Claim Language	Infringement Evidence
		<p>positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • US9738887 (Exhibit 31) at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • US9738887 (Exhibit 31) at 15:31-35 (“The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.”) • US9738887 (Exhibit 31) at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which

Claim	Claim Language	Infringement Evidence
		<p>the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> US9738887 (Exhibit 31) at Figs. 1J and 1K:  <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p>
20(d)	amplifying polynucleotides contained with the plurality of samples in the plurality of PCR reaction zones while thermal cycling the PCR reaction zones	<p>The accused workflow comprises amplifying polynucleotides contained with the plurality of samples in the plurality of PCR reaction zones while thermal cycling the PCR reaction zones.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.” 

Claim	Claim Language	Infringement Evidence
		<p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

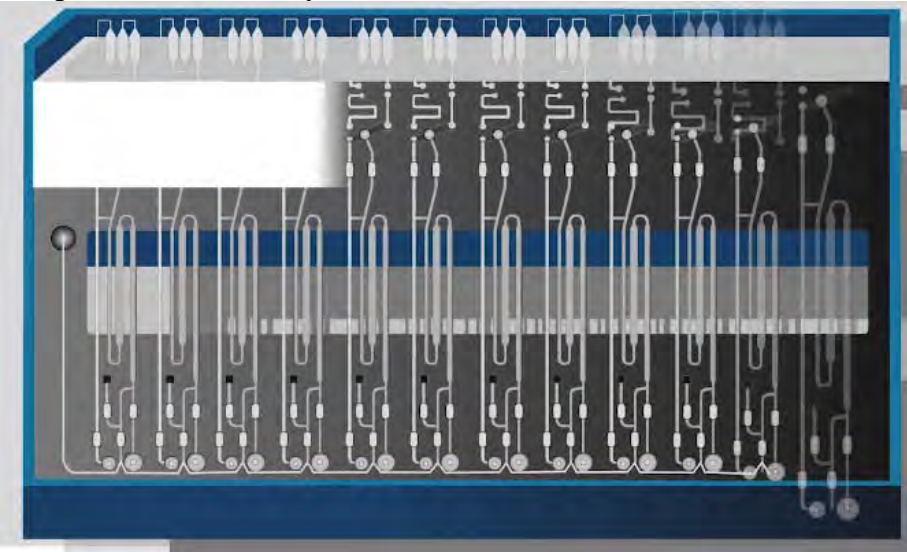
Claim	Claim Language	Infringement Evidence
		<div data-bbox="890 233 1793 776" data-label="Image"> </div> <ul style="list-style-type: none"> <li data-bbox="842 786 1923 1000"> <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p> <div data-bbox="905 1039 1612 1383" data-label="Image"> </div>

Claim	Claim Language	Infringement Evidence
		<p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module

Claim	Claim Language	Infringement Evidence
		<p>comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip,

Claim	Claim Language	Infringement Evidence
		<p>and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of

Claim	Claim Language	Infringement Evidence
		<p>nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)
20(e)	and maintaining a substantially uniform temperature throughout each PCR reaction zone during each cycle,	The accused workflow comprises amplifying polynucleotides contained with the plurality of samples in the plurality of PCR reaction zones while thermal cycling the PCR reaction zones and maintaining a substantially uniform temperature throughout each PCR reaction zone during each cycle

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		<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

Claim	Claim Language	Infringement Evidence
		<div data-bbox="905 228 1612 574" data-label="Image"> </div> <p data-bbox="793 646 1877 719"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="842 727 1921 1052" style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p data-bbox="793 1092 1108 1125">US9050594 (Exhibit 24)</p> <ul data-bbox="842 1133 1921 1414" style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended


Claim	Claim Language	Infringement Evidence
		<p>configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.

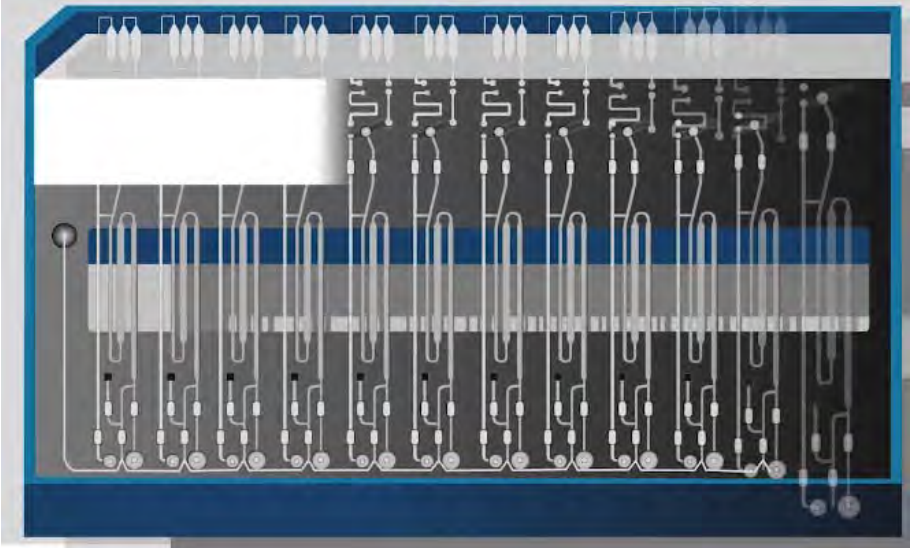
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of

Claim	Claim Language	Infringement Evidence
		<p>the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)</p> <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • U.S. Patent No. 9,499,896 at 2:33-48 “The system 100 functions to enable rapid thermal cycling of samples while providing uniform heating and preventing signal drift. In specific applications, the system 100 can be used to rapidly and controllably thermocycle nucleic acid samples during performance of molecular diagnostic amplification techniques (e.g., PCR, RT-PCR), signal amplification techniques (e.g., bDNA, hybrid capture), and analytical techniques (e.g., gel electrophoresis, mass spectrometry). The system 100 can also provide rapid thermocycling without significant power requirements, ensure a closer correlation between the actual heating temperature and the temperature set-point by implementing an integrated heater-sensor die, and controllably and individually heat small sample volumes (e.g., picoliters, nanoliters) based upon a microfabrication technique that also enables mass production of the system 100.”) • U.S. Patent No. 9,499,896 at 2:61-3:3 (“The set of heater-sensor dies 110 functions to controllably heat individual sample volumes. Preferably, each heater sensor die 111 is a thin-film die that can be deposited onto another substrate (e.g., silicon, glass substrate) that can be packaged onto an electronics substrate 140 (e.g., printed circuit board, PCB); however, each heater-sensor die 111 can alternatively comprise any suitable geometry and/or configuration that enables controlled, uniform, and rapid heating of a detection chamber in thermal communication with the heater-sensor die 111.”) • U.S. Patent No. 9,499,896 at 3:23-27 (“Preferably, each heater-sensor die 111 in the set of heater sensor dies 110 comprises an assembly including: a first insulating layer 112a that functions to provide an insulating barrier to isolate the heaters and sensors and a heating region 113 that functions to provide uniform sample heating.”)
20(f)	at least one PCR reaction zone separately thermally controllable from another PCR reaction zone.	<p>The accused workflow comprises at least one PCR reaction zone separately thermally controllable from another PCR reaction zone.</p> <p><i>NeuMoDx™ Molecular Systems, NEUMODx, http://www.neumodx.com/our-solutions/,</i></p>

Claim	Claim Language	Infringement Evidence
		<p>last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieveing [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

Claim	Claim Language	Infringement Evidence
		 <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of

Claim	Claim Language	Infringement Evidence
		<p>up to eight hours.” <i>Id.</i> at 0:00-0:18</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

Claim	Claim Language	Infringement Evidence
		<div data-bbox="905 233 1612 574" data-label="Image"> </div> <p data-bbox="793 651 1108 683">US9403165 (Exhibit 27)</p> <ul data-bbox="842 691 1919 1414" style="list-style-type: none"> • Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. • Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the

Claim	Claim Language	Infringement Evidence
		<p>second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module,


Claim	Claim Language	Infringement Evidence
		<p>configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of

Claim	Claim Language	Infringement Evidence
		<p>nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but


Claim	Claim Language	Infringement Evidence
		alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)

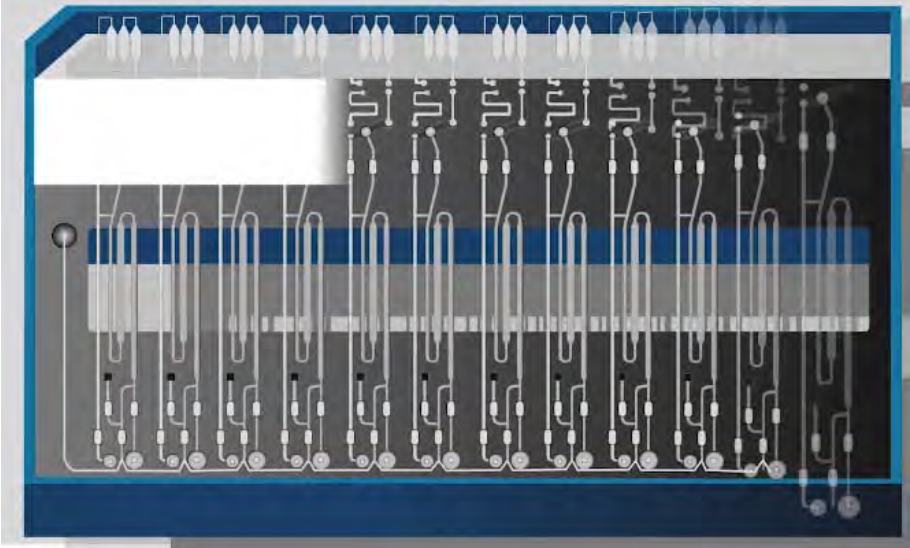
Exhibit 38

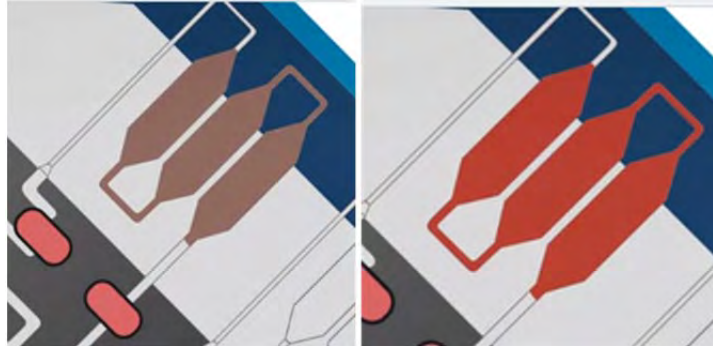
U.S. Patent No. 8,415,103 Infringement Chart

Claim	Claim Language	Infringement Evidence
1(a)	A method of carrying out amplification independently on a plurality of polynucleotide-containing samples, the method comprising:	<p data-bbox="793 375 1850 440">To the extent the preamble is limiting, the accused workflow includes carrying out amplification independently on a plurality of polynucleotide-containing samples.</p> <p data-bbox="793 477 1902 548"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p> <div data-bbox="800 553 1843 1360">  <div data-bbox="821 1214 1270 1349"> <p>#500200 NeuMoDx™ 96 Molecular System</p> </div> <div data-bbox="1373 1214 1822 1349"> <p>#500100 NeuMoDx™ 288 Molecular System</p> </div> </div>

Claim	Claim Language	Infringement Evidence
		<p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

Claim	Claim Language	Infringement Evidence
		 <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of

Claim	Claim Language	Infringement Evidence
		<p>up to eight hours.” <i>Id.</i> at 0:00-0:18</p> <ul style="list-style-type: none"> <p>“This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59</p>  <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p>

Claim	Claim Language	Infringement Evidence										
		<div></div> <p>“Patents”, http://www.neumodx.com/patents/, demonstrating that NeuMoDx marks its products with US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; 9,452,430; 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; 10,010,888; 9,382,532; 9,540,636; 9,499,896; 9,539,576; 9,637,775; and 10,093,963 (Exhibit 15)</p> <h2>PATENTS</h2> <table><tr><th>Product</th><th>Patents</th></tr><tr><td>CARTRIDGE</td><td>US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.</td></tr><tr><td>P02 (overall system and method)</td><td>US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.</td></tr><tr><td>EXTRACTION PLATE</td><td>US Patent Nos. 9,382,532; and 9,540,636.</td></tr><tr><td>XPCR MODULE</td><td>US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.</td></tr></table> <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none">Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate	Product	Patents	CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.	P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.	EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.	XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.
Product	Patents											
CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.											
P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.											
EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.											
XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.											


Claim	Claim Language	Infringement Evidence
		<p>is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate.</p> <ul style="list-style-type: none"> Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the

Claim	Claim Language	Infringement Evidence
		<p>actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the

Claim	Claim Language	Infringement Evidence
		<p>emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter.</p> <ul style="list-style-type: none"> • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”) • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge

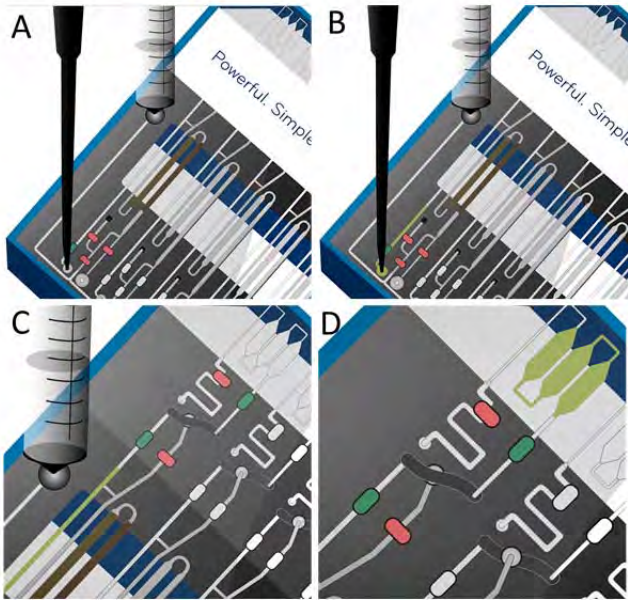
Claim	Claim Language	Infringement Evidence
		<p>210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).")</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 10:63-65 ("The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.") • U.S. Patent No. 9,050,594 at 11:56-58 ("The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.") • U.S. Patent No. 9,050,594 at 29:44-47 ("In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.")
1(b)	introducing the plurality of samples separately into a microfluidic cartridge;	<p>The accused workflow includes introducing the plurality of samples separately into a microfluidic cartridge.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to "VIDEO NeuMoDx™ WORKFLOW" hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • "The liquid handling robot aspirates the PCR-ready solution and transfers it back to the cartridge where it dispenses into the same P-port from which the sample was aspirated." <i>Id.</i> at 3:47-3:57

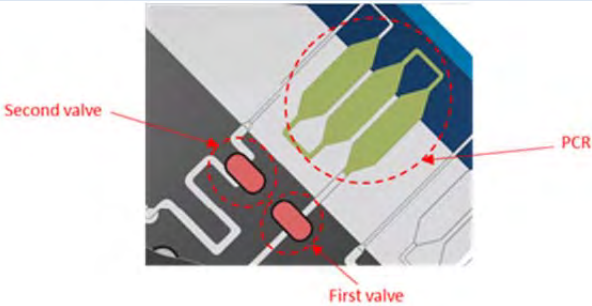
Claim	Claim Language	Infringement Evidence
		

Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 935 1113 967">US9101930 (Exhibit 25)</p> <ul data-bbox="848 976 1921 1408" style="list-style-type: none"> • Claim 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising: a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer; a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, are each at least partially separated from the corrugated surface of the

Claim	Claim Language	Infringement Evidence
		<p>intermediate substrate by an elastomeric layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate.</p> <ul style="list-style-type: none"> Claim 11. The cartridge of claim 10, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second

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		<p>sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer.


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1(c)	isolating the samples in the microfluidic cartridge;	<p>The accused workflow includes isolating the samples in the microfluidic cartridge.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 

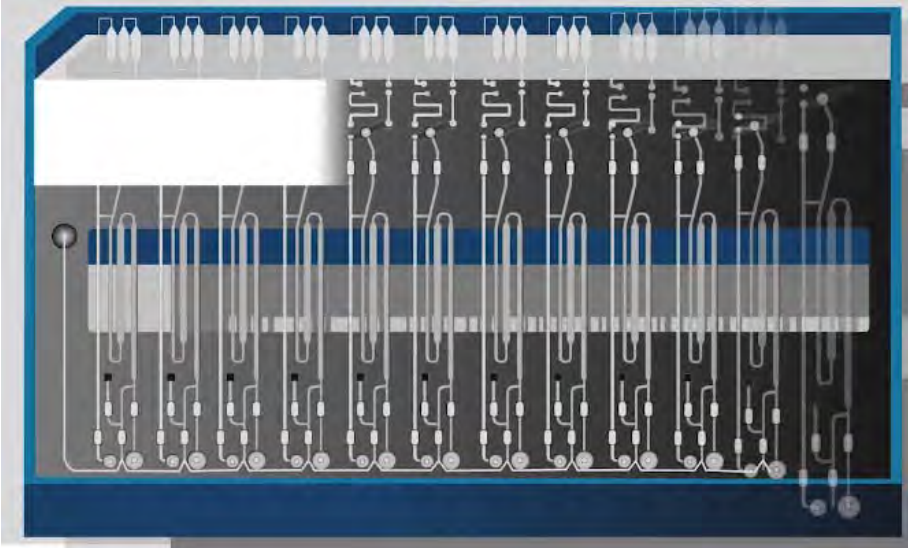
Claim	Claim Language	Infringement Evidence
		 <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> • Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads. • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to

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		<p>facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber.</p> <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined

Claim	Claim Language	Infringement Evidence
		<p>between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured

Claim	Claim Language	Infringement Evidence
		<p>to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at Figs. 1J and 1K: <div data-bbox="961 423 1745 711"> <p>FIG. 1J</p> </div> <div data-bbox="961 727 1745 1015"> <p>FIG. 1K</p> <p>○ OCCLUDED</p> </div> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)

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1(d)	placing the microfluidic cartridge in thermal communication with an array of independent heaters; and	<p>The accused workflow includes placing the microfluidic cartridge in thermal communication with an array of independent heaters.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.”

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		<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26


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		<div data-bbox="905 228 1612 574"> </div> <p data-bbox="793 651 1110 683">US9050594 (Exhibit 24)</p> <ul data-bbox="842 691 1919 1421" style="list-style-type: none"> <li data-bbox="842 691 1919 1198">• Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. <li data-bbox="842 1206 1919 1421">• Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment

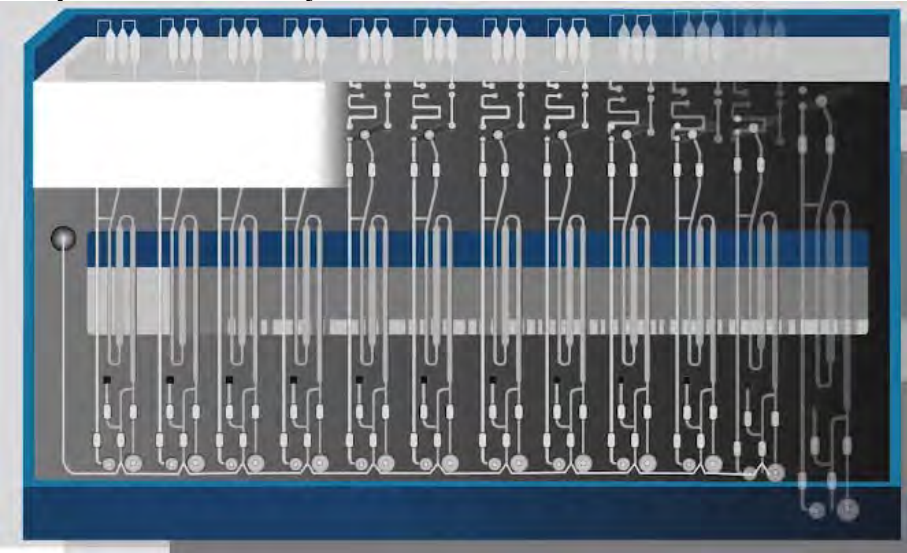
Claim	Claim Language	Infringement Evidence
		<p>configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer.</p> <ul style="list-style-type: none"> • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater

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		<p>is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”) • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection

Claim	Claim Language	Infringement Evidence
		<p>chambers 213 within a microfluidic cartridge 210.”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,050,594 at 29:44-47 (“In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.”) <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers. U.S. Patent No. 9,499,896 at 12:15-20 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies 110.”)
1(e)	amplifying polynucleotides in the plurality of samples by	The accused workflow includes amplifying polynucleotides in the plurality of samples by independent application of successive temperature cycles to each sample.

Claim	Claim Language	Infringement Evidence
	independent application of successive temperature cycles to each sample.	<p><i>NeuMoDx™ Molecular Systems</i>, NEUMODx, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieveing [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

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		 <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of

Claim	Claim Language	Infringement Evidence
		<p>up to eight hours.” <i>Id.</i> at 0:00-0:18</p> <ul style="list-style-type: none"> <p>“This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59</p>  <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p>


Claim	Claim Language	Infringement Evidence
		<div data-bbox="905 233 1612 574" data-label="Image"> </div> <p data-bbox="793 651 1108 683">US9403165 (Exhibit 27)</p> <ul data-bbox="842 691 1919 1421" style="list-style-type: none"> <li data-bbox="842 691 1919 1162">• Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. <li data-bbox="842 1170 1919 1421">• Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the

Claim	Claim Language	Infringement Evidence
		<p>second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module,


Claim	Claim Language	Infringement Evidence
		<p>configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of

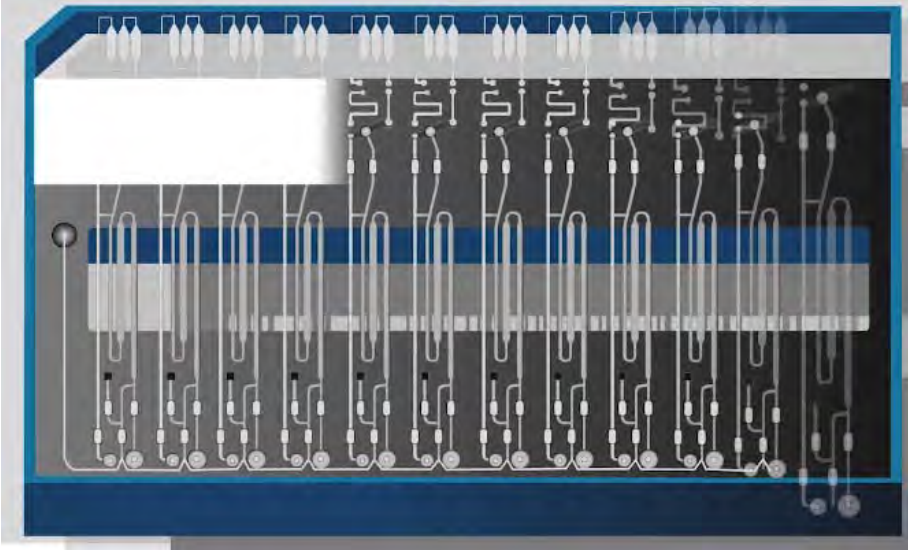
Claim	Claim Language	Infringement Evidence
		<p>nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 29:44-47 (“In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.”) <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface

Claim	Claim Language	Infringement Evidence
		<p>with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,539,576 at 9:8-12 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies no.”) • U.S. Patent No. 9,539,576 at 12:59-64 (“Upon completion of Block S240, individual heater-sensor dies of the set of heater-sensor dies can be coupled to one or multiple electronics substrates in order to provide uniform heating of

Claim	Claim Language	Infringement Evidence
		individual sample containers with independent control of heating parameters provided at each of the set of heater-sensor dies.”)
15(a)	A method of carrying out amplification independently on a plurality of polynucleotide-containing samples, the method comprising:	<p>To the extent the preamble is limiting, the accused workflow includes carrying out amplification independently on a plurality of polynucleotide-containing samples.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-products/, last visited June 5, 2019 (Exhibit 12)</p> 

Claim	Claim Language	Infringement Evidence
		<p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieveing [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent

Claim	Claim Language	Infringement Evidence
		<p data-bbox="890 233 1423 266">sample processing and real-time PCR.”</p> <div data-bbox="890 305 1646 776">  </div> <p data-bbox="800 834 1675 867">40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul data-bbox="842 873 1919 1159" style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p data-bbox="793 1203 1902 1344"><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul data-bbox="842 1351 1860 1414" style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random


Claim	Claim Language	Infringement Evidence
		<p>access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26


Claim	Claim Language	Infringement Evidence
		<div data-bbox="905 233 1612 574" data-label="Image"> </div> <p data-bbox="793 651 1108 683">US9403165 (Exhibit 27)</p> <ul data-bbox="842 691 1919 1414" style="list-style-type: none"> • Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. • Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the

Claim	Claim Language	Infringement Evidence
		<p>second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module,

Claim	Claim Language	Infringement Evidence
		<p>configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of

Claim	Claim Language	Infringement Evidence
		<p>nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 29:44-47 (“In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.”)
15(b)	introducing the plurality of samples in to a microfluidic cartridge,	The accused workflow includes introducing the plurality of samples in to a microfluidic cartridge.


Claim	Claim Language	Infringement Evidence
		<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The liquid handling robot aspirates the PCR-ready solution and transfers it back to the cartridge where it dispenses into the same P-port from which the sample was aspirated.” <i>Id.</i> at 3:47-3:57 

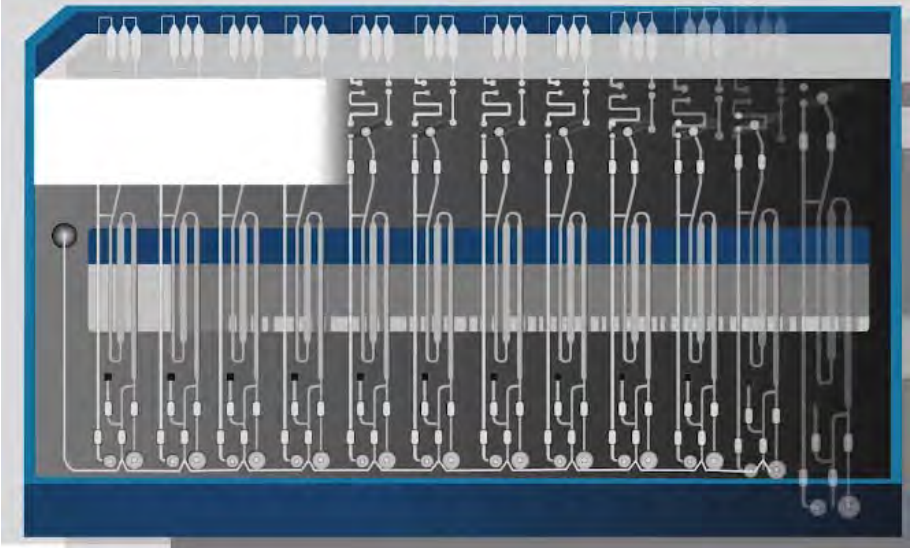
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 935 1115 967">US9101930 (Exhibit 25)</p> <ul data-bbox="842 976 1923 1408" style="list-style-type: none"> • Claim 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising: a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer; a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, are each at least partially separated from the corrugated surface of the

Claim	Claim Language	Infringement Evidence
		<p>intermediate substrate by an elastomeric layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate.</p> <ul style="list-style-type: none"> Claim 11. The cartridge of claim 10, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second

Claim	Claim Language	Infringement Evidence
		<p>sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. •

Claim	Claim Language	Infringement Evidence
15(c)	wherein the cartridge has a plurality of reaction chambers configured to permit thermal cycling of the plurality of samples independently of one another;	<p>In the accused workflow, the cartridge has a plurality of reaction chambers configured to permit thermal cycling of the plurality of samples independently of one another.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.” • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> • Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

Claim	Claim Language	Infringement Evidence
		 <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of

Claim	Claim Language	Infringement Evidence
		<p>up to eight hours.” <i>Id.</i> at 0:00-0:18</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26

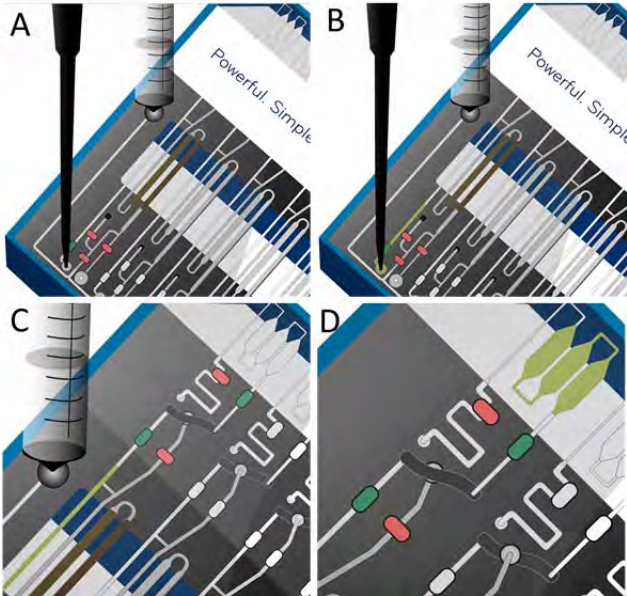
Claim	Claim Language	Infringement Evidence
		<div data-bbox="905 233 1612 574" data-label="Image"> </div> <p data-bbox="793 651 1115 683">US9403165 (Exhibit 27)</p> <ul data-bbox="842 691 1919 1421" style="list-style-type: none"> <li data-bbox="842 691 1919 1162">• Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. <li data-bbox="842 1170 1919 1421">• Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the

Claim	Claim Language	Infringement Evidence
		<p>second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module,

Claim	Claim Language	Infringement Evidence
		<p>configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of

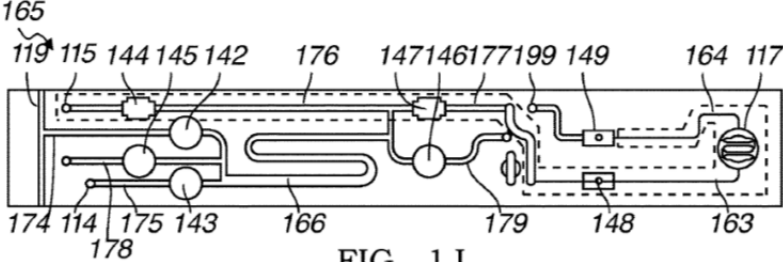
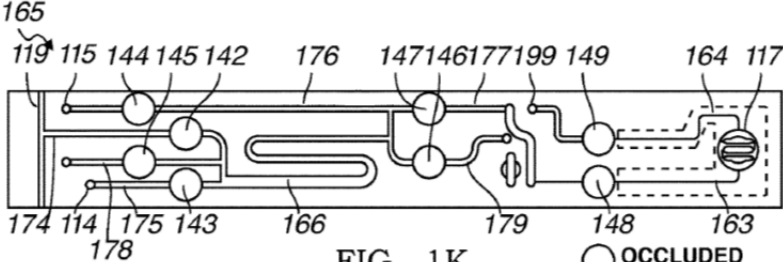
Claim	Claim Language	Infringement Evidence
		<p>nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 29:44-47 (“In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.”) <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface

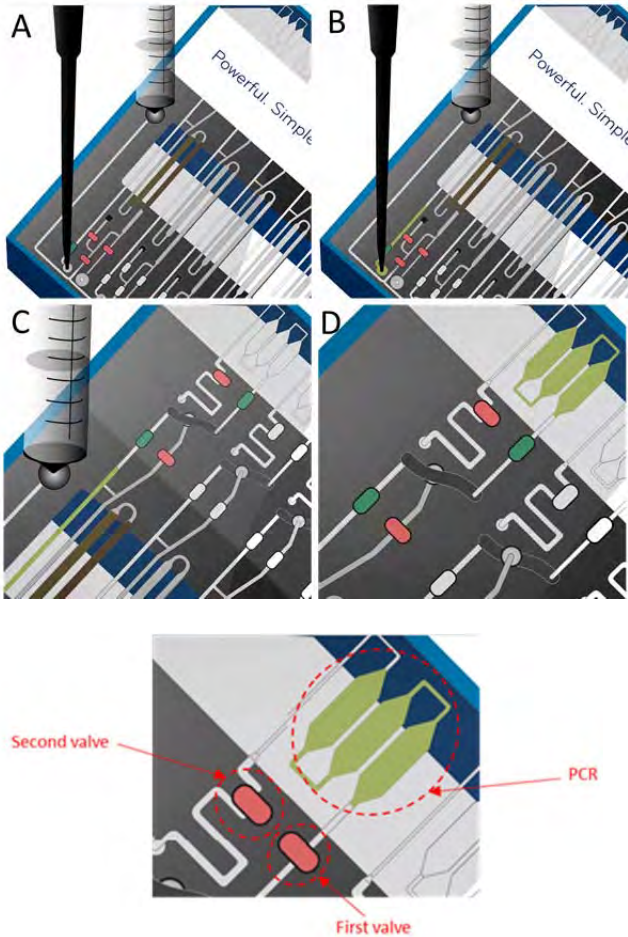
Claim	Claim Language	Infringement Evidence
		<p>with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,539,576 at 9:8-12 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies no.”) • U.S. Patent No. 9,539,576 at 12:59-64 (“Upon completion of Block S240, individual heater-sensor dies of the set of heater-sensor dies can be coupled to one or multiple electronics substrates in order to provide uniform heating of

Claim	Claim Language	Infringement Evidence
		individual sample containers with independent control of heating parameters provided at each of the set of heater-sensor dies.”)
15(d)	moving the plurality of samples independently of one another into the respective plurality of reaction chambers;	<p>The accused workflow includes moving the plurality of samples independently of one another into the respective plurality of reaction chambers.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 

Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second

Claim	Claim Language	Infringement Evidence
		<p>branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • US Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • US Patent No. 9,738,887 at 15:31-35 (“The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.”) • US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth

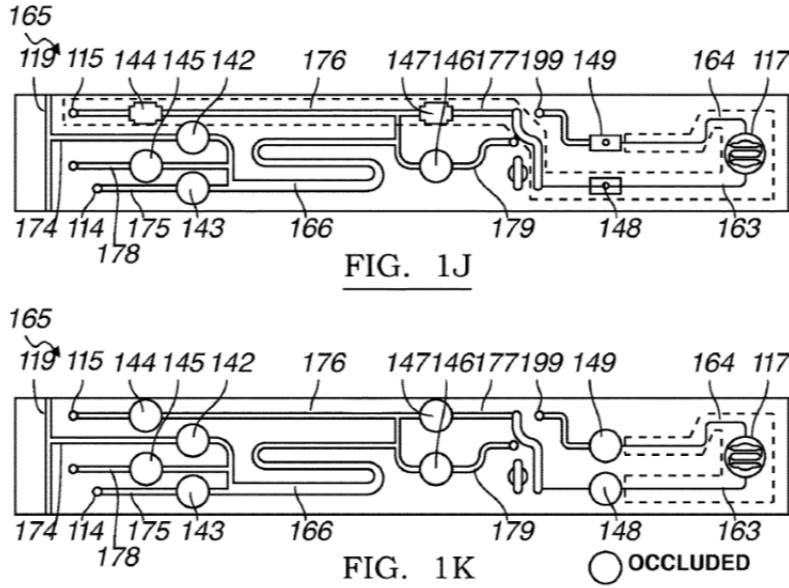
Claim	Claim Language	Infringement Evidence
		<p>truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at Figs. 1J and 1K:  <p style="text-align: center;">FIG. 1J</p>  <p style="text-align: center;">FIG. 1K</p> <p style="text-align: right;">○ OCCLUDED</p>
15(e)	isolating the samples within the plurality of reaction chambers;	<p>The accused workflow includes isolating the samples within the plurality of reaction chambers.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the

Claim	Claim Language	Infringement Evidence
		<p data-bbox="890 235 1822 305">cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08</p>  <p data-bbox="793 1289 1276 1321">US9339812 (Exhibit 26) (Exhibit 26)</p> <ul data-bbox="842 1328 1843 1396" style="list-style-type: none"> • Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions

Claim	Claim Language	Infringement Evidence
		<p>defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads.</p> <ul style="list-style-type: none"> • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber. <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection

Claim	Claim Language	Infringement Evidence
		<p>chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
15(f)	placing the microfluidic cartridge in thermal communication with an array of independent heaters; and	<p>The accused workflow includes placing the microfluidic cartridge in thermal communication with an array of independent heaters.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.” <div data-bbox="888 342 1646 813"> </div> <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>


Claim	Claim Language	Infringement Evidence
		<div data-bbox="890 233 1793 776" data-label="Image"> </div> <ul style="list-style-type: none"> <li data-bbox="842 786 1923 1000"> <p>“A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p> <div data-bbox="905 1039 1612 1383" data-label="Image"> </div>

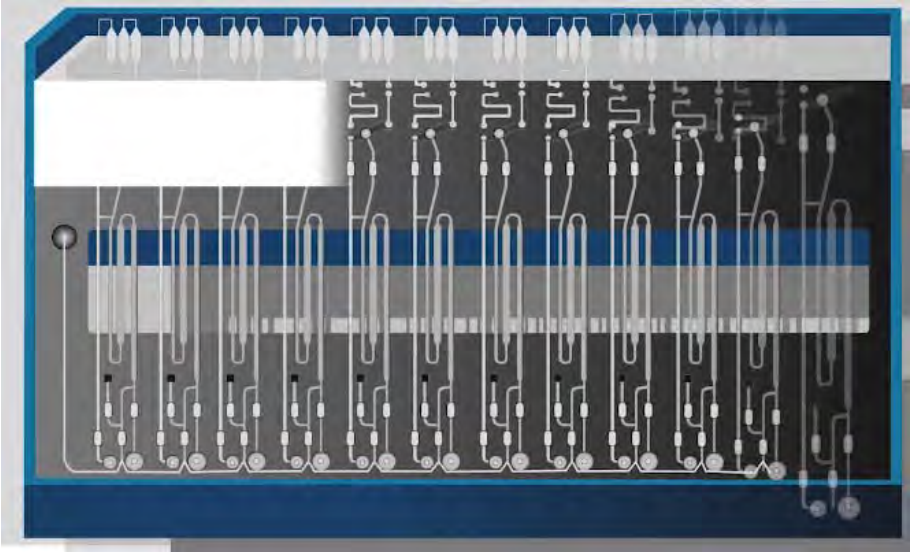
Claim	Claim Language	Infringement Evidence
		<p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic

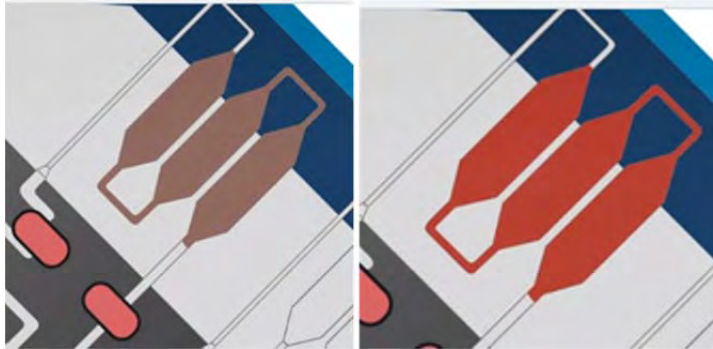
Claim	Claim Language	Infringement Evidence
		<p>acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads,

Claim	Claim Language	Infringement Evidence
		<p>isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 29:44-47 (“In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.”) <p>US9499896 (Exhibit 28)</p> <ul style="list-style-type: none"> • Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising: an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first

Claim	Claim Language	Infringement Evidence
		<p>insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements coupled to a second substrate surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein the set of heater-sensor dies is in thermal communication with a set of detection chambers.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,499,896 at 12:15-20 (“Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies 110.”)
15(g)	amplifying polynucleotides contained within the plurality of samples, by application of successive temperature cycles independently to the reaction chambers.	<p>The accused workflow includes amplifying polynucleotides contained within the plurality of samples, by application of successive temperature cycles independently to the reaction chambers.</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result” platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.”

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p><i>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</i></p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a

Claim	Claim Language	Infringement Evidence
		<p>combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.”</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “The NeuMoDx Molecular N96 and N288 are fully automated sample to result molecular diagnostics platforms. They provide continuous random access processing with initial results in one hour and operator walk away time of up to eight hours.” <i>Id.</i> at 0:00-0:18 • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the

Claim	Claim Language	Infringement Evidence
		<p>cartridge into three thin PCR chambers and the amplification process begins. During a series of independent heat on-heat off sequences, an optical scanner measures the level of fluorescence emitted, and converts it into the qualitative or quantitative results which are displayed as amplification curves for analysis by the laboratorian.” <i>Id.</i> at 3:58-4:26</p>  <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. Claim 10. The cartridge of claim 8, wherein the first layer is a unitary

Claim	Claim Language	Infringement Evidence
		<p>construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample. • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer.

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module. • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent


Claim	Claim Language	Infringement Evidence
		<p>mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids.”) U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) U.S. Patent No. 9,050,594 at 29:44-47 (“In embodiments wherein multiple

Claim	Claim Language	Infringement Evidence
		<p>heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample.”)</p> <p>US9539576 (Exhibit 29)</p> <ul style="list-style-type: none"> Claim 1. A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element; an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller; a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points. U.S. Patent No. 9,539,576 at 9:8-12 (“Furthermore, the controller 165 can be

Claim	Claim Language	Infringement Evidence
		<p>configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies no.")</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,539,576 at 12:59-64 ("Upon completion of Block S240, individual heater-sensor dies of the set of heater-sensor dies can be coupled to one or multiple electronics substrates in order to provide uniform heating of individual sample containers with independent control of heating parameters provided at each of the set of heater-sensor dies.")

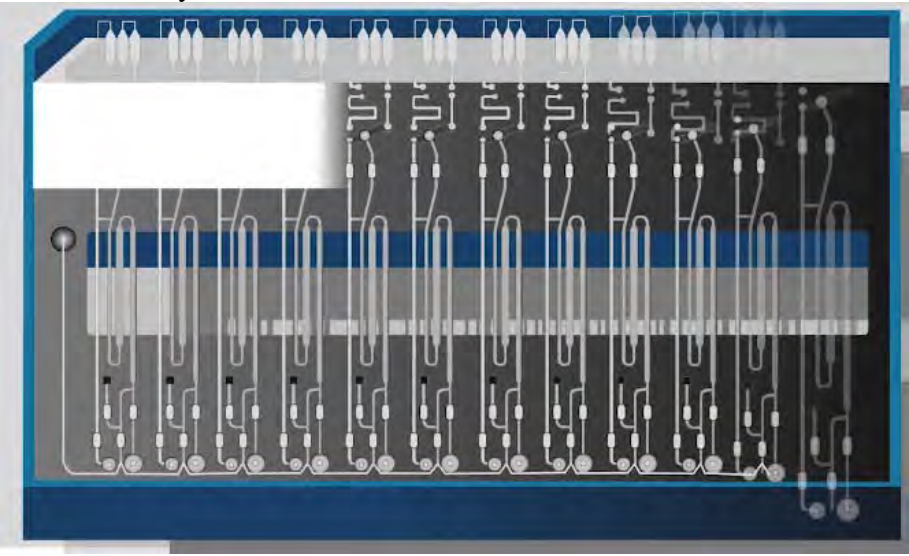
EXHIBIT 39

U.S. Patent No. 8,709,787 Infringement Chart

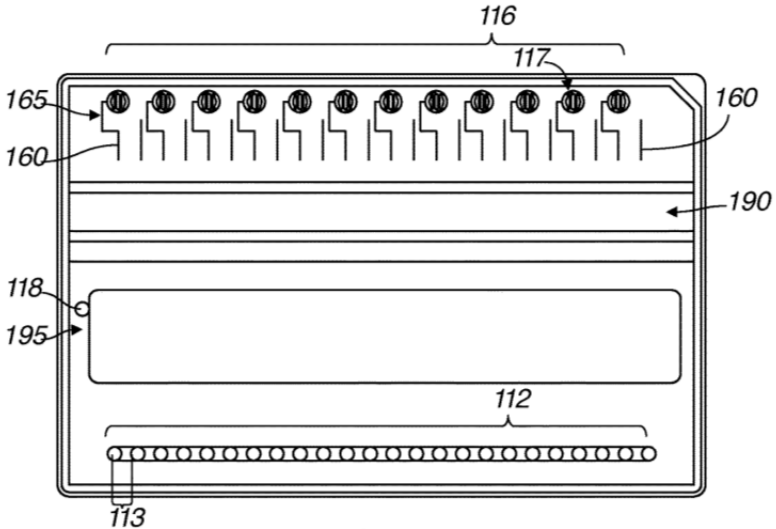
Claim	Claim Language	Infringement Evidence
10(a)	A microfluidic substrate, comprising:	<p>To the extent the preamble is limiting, the accused product is a microfluidic substrate.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample

Claim	Claim Language	Infringement Evidence
		<p>microfluidic cartridge.”</p> <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” • “The NeuMoDx™ 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” • “The NeuMoDx™ 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx™ 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-288/, last visited June 3, 2019 (Exhibit 13)</p> <ul style="list-style-type: none"> • “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/product/neumodx-96/, last visited June 3, 2019 (Exhibit 14)</p>


Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> “FEATURES AND BENEFITS... Fluorescence detection at five wavelengths enabling multiplexed amplification reactions... Real-time detection of products of amplification.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>0600101_Rev-D-IFU-NeuMoDx-RELEASE-Solution-US-ONLY-FINAL-25Oct2018.pdf (Exhibit 20)</p> <ul style="list-style-type: none"> “NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE... The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) and the dried Master Mix contained in a NeuMoDx test strip. The System then dispenses the prepared RT-PCR-ready mixture into the NeuMoDx Cartridge where Real-Time PCR occurs.” <p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO </p>

Claim	Claim Language	Infringement Evidence
		<p>NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 <p>“Patents”, http://www.neumodx.com/patents/, demonstrating that NeuMoDx marks its products with US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; 9,452,430; 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; 10,010,888; 9,382,532; 9,540,636; 9,499,896; 9,539,576; 9,637,775; and 10,093,963. (Exhibit 15)</p>

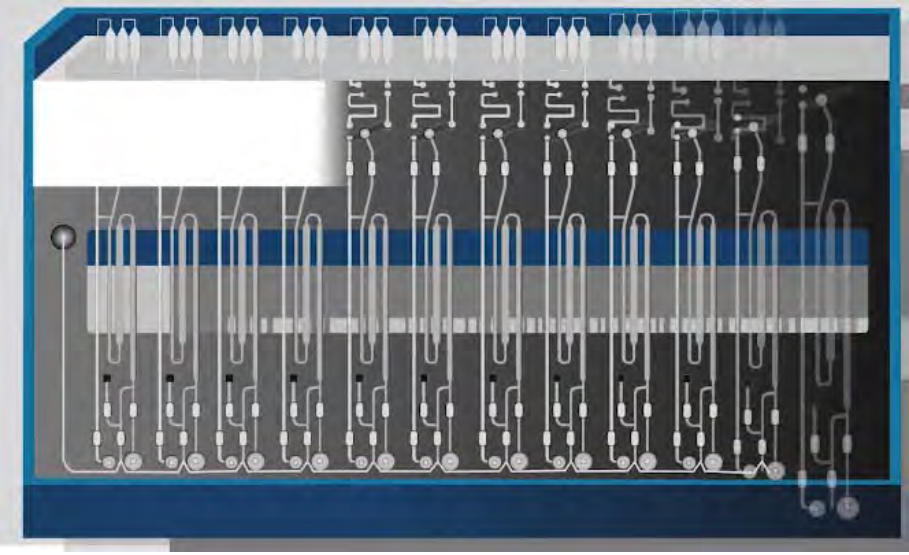
Claim	Claim Language	Infringement Evidence										
		<div>PATENTS</div> <table><tr><th>Product</th><th>Patents</th></tr><tr><td>CARTRIDGE</td><td>US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.</td></tr><tr><td>P02 (overall system and method)</td><td>US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.</td></tr><tr><td>EXTRACTION PLATE</td><td>US Patent Nos. 9,382,532; and 9,540,636.</td></tr><tr><td>XPCR MODULE</td><td>US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.</td></tr></table> <div>US9738887 (Exhibit 31)</div> <ul style="list-style-type: none">Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent port, the fluid port, and the detection chamber.Claim 11. The cartridge of claim 1, wherein the detection chamber comprises a first, a second, and a third detection chamber segment wherein each of the first, the second, and the third detection chamber segment is a broad chamber of which a projection onto a plane is substantially rectangular, wherein a first end of the second detection chamber segment is connected to the first detection chamber segment by a first narrow fluidic channel, and wherein a second end of the second detection chamber segment is connected to the third detection	Product	Patents	CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.	P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.	EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.	XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.
Product	Patents											
CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.											
P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.											
EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.											
XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.											

Claim	Claim Language	Infringement Evidence
		<p>chamber segment by a second narrow fluidic channel.</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at FIG. 1A:  <p style="text-align: center;">FIG. 1A</p> U.S. Patent No. 9,738,887 at Abstract (“A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste

Claim	Claim Language	Infringement Evidence
		<p>chamber, and to pass through the vent region.”)</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 2:36-3:5. (“As shown in FIGS. 1A-1C, an embodiment of a microfluidic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample port-reagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; and a set of fluidic pathways 160, each formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140... In a specific application, the microfluidic cartridge 100 can be used to facilitate a PCR procedure for analysis of a sample containing nucleic acids.”) US Patent No. 9,738,887 at 13:7-18. (“The top layer 110 of an embodiment of the microfluidic cartridge 100 functions to accommodate elements involved in performing a molecular diagnostic procedure (e.g. PCR), such that a sample containing nucleic acids, passing through the cartridge, can be manipulated by the elements involved in performing the molecular diagnostic procedure. The top layer 110 is preferably composed of a structurally rigid/stiff material with low autofluorescence, such that the top layer 110 does not interfere with sample detection by fluorescence or chemiluminescence techniques, and an appropriate glass transition temperature and chemical compatibility for PCR or other amplification techniques.”) US Patent No. 9,738,887 at 13:35-42. (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) US Patent No. 9,738,887 at 15:29-39 (“The segments may be arranged in at

Claim	Claim Language	Infringement Evidence
		<p>least one of several configurations to facilitate isolation, processing, and amplification of a nucleic acid sample ...").</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 23:20-24 ("The top layer 110 of the specific embodiment of the microfluidic cartridge 100 functions preferably as described in Section 1.1, and is composed of polypropylene with low autofluorescence and a glass transition temperature suitable for PCR.")
10(b)	a plurality of sample lanes,	<p>The accused microfluidic substrate comprises a plurality of sample lanes,</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf</i> (Exhibit 18)</p> <ul style="list-style-type: none"> Describing "...microfluidic cartridges capable of performing independent sample processing and real-time PCR."  <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul style="list-style-type: none"> "NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, "sample to result"

Claim	Claim Language	Infringement Evidence
		<p>platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.”</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. <p><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul style="list-style-type: none"> • “NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.” <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System.... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.”

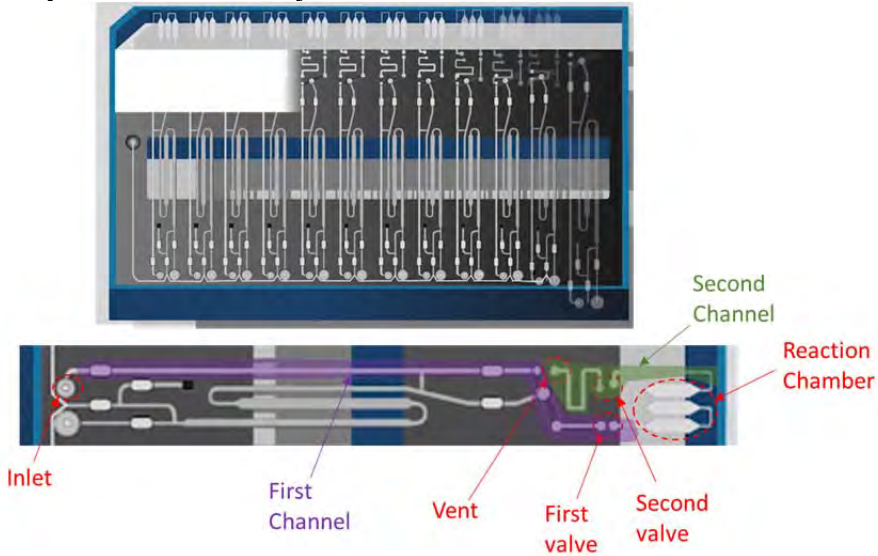
Claim	Claim Language	Infringement Evidence
		<p>K173725.pdf (Exhibit 23)</p> <ul style="list-style-type: none"> “510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY AND INSTRUMENT COMBINATION TEMPLATE... Test Principle... After reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Amplification and detection of the control and target DNA sequences occur in PCR chamber.” <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the


Claim	Claim Language	Infringement Evidence
		<p>cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08</p> <p>US9403165 (Exhibit 27)</p> <ul style="list-style-type: none"> • Claim 8. A cartridge for processing a sample, the cartridge comprising: a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate. • Claim 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port. <p>US9050594 (Exhibit 24)</p> <ul style="list-style-type: none"> • Claim 1: A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and a molecular diagnostic module, configured to process at

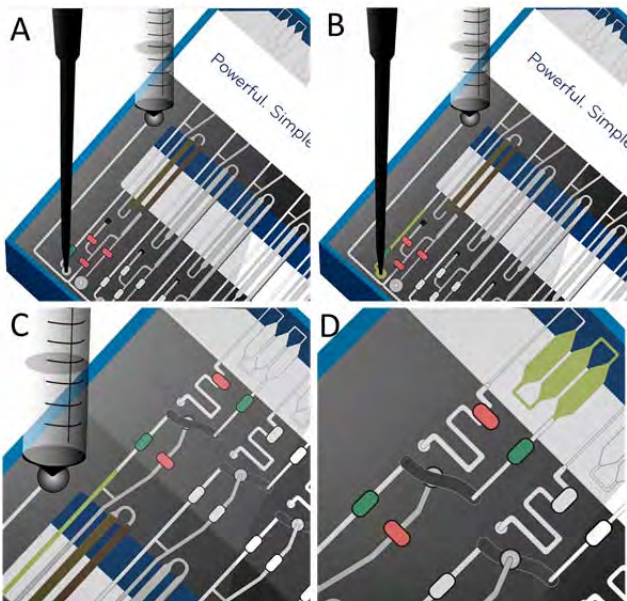
Claim	Claim Language	Infringement Evidence
		<p>least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic beads, wherein the molecular diagnostic module comprises: a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform, a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and a cam card contacting a set of pins, wherein the extended configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.</p> <ul style="list-style-type: none"> • Claim 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer. • Claim 16. A system for processing and detecting nucleic acids, comprising: a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample; an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume to produce a nucleic acid-reagent mixture; a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, and analyze the nucleic acid-reagent mixture from the assay strip, wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and a liquid handling system configured to transfer the magnetic bead-

Claim	Claim Language	Infringement Evidence
		<p>sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.</p> <ul style="list-style-type: none"> • Claim 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture, through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter. • Claim 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acid-reagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater. • U.S. Patent No. 9,050,594 at Abstract (“A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to

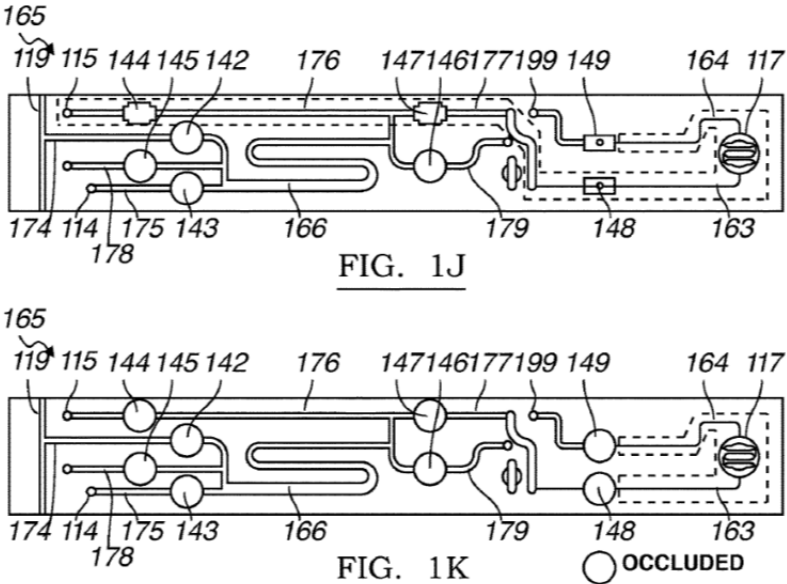
Claim	Claim Language	Infringement Evidence
		<p>facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,050,594 at 9:4-21 (“The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210... The vertical displacement also allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the nucleic acid (e.g. PCR).”) • U.S. Patent No. 9,050,594 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”) • U.S. Patent No. 9,050,594 at 11:56-58 (“The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210.”) • U.S. Patent No. 9,050,594 at 31:32043 (“Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.”)
10(c)	wherein each of the plurality of sample lanes comprises a microfluidic network having, in	In the accused microfluidic substrate, each of the plurality of sample lanes comprises a microfluidic network having, in fluid communication with one another, an inlet.

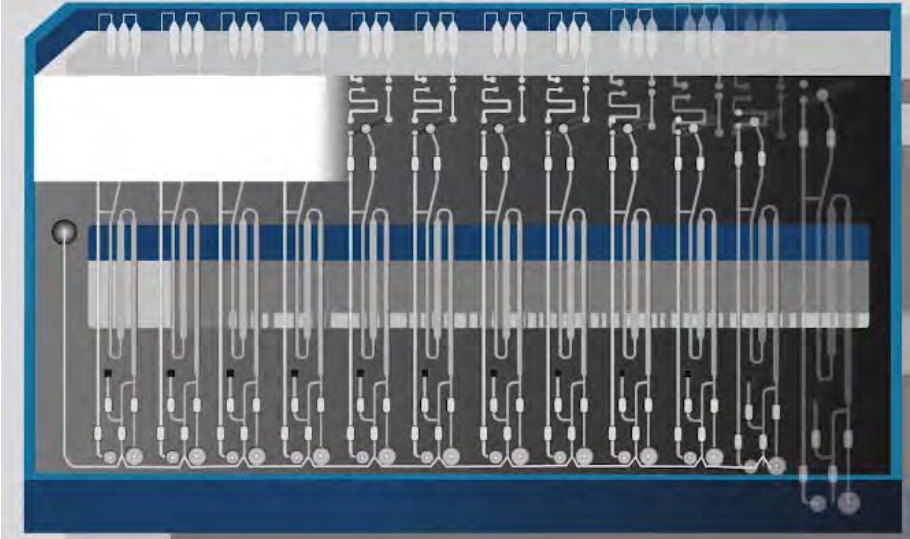
Claim	Claim Language	Infringement Evidence
	fluid communication with one another: an inlet;	<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> “The liquid handling robot aspirates the PCR-ready solution and transfers it back to the cartridge where it dispenses into the same P-port from which the sample was aspirated.” <i>Id.</i> at 3:47-3:57

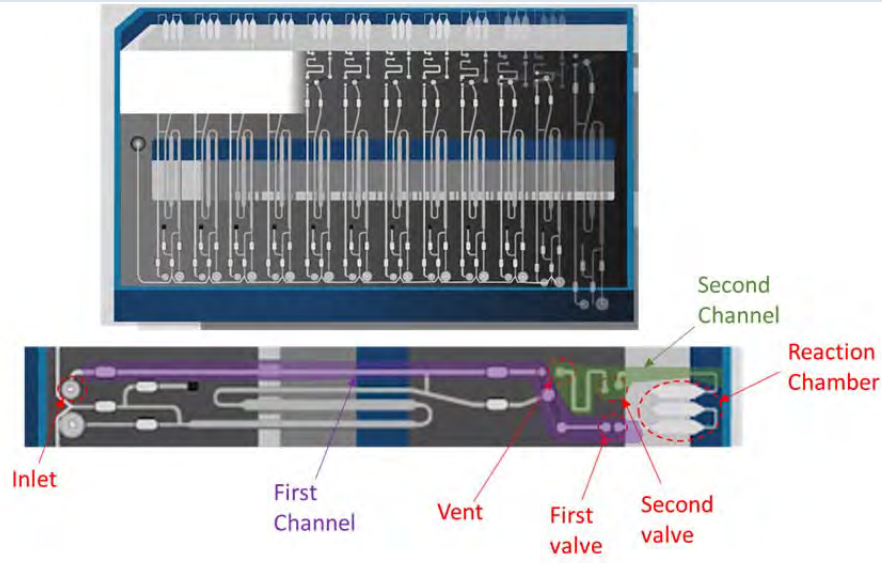
Claim	Claim Language	Infringement Evidence
		<div data-bbox="890 228 1814 937"></div> <ul style="list-style-type: none">• “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

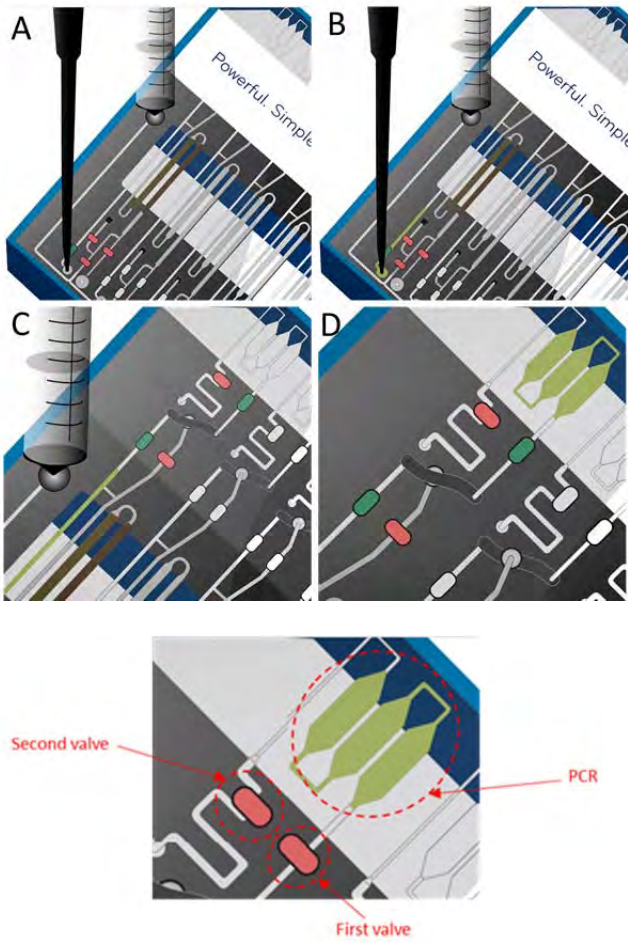
Claim	Claim Language	Infringement Evidence
		 <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.”) • U.S. Patent No. 9,738,887 at 15:31-35 (“The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.”) • U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142,

Claim	Claim Language	Infringement Evidence
		<p>144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p>
10(d)	a first valve and a second valve;	<p>In the accused microfluidic substrate, each of the plurality of sample lanes comprises a microfluidic network having, in fluid communication with one another, a first valve and a second valve.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

Claim	Claim Language	Infringement Evidence
		 <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08 (see below, with elements of the accused product marked for reference)

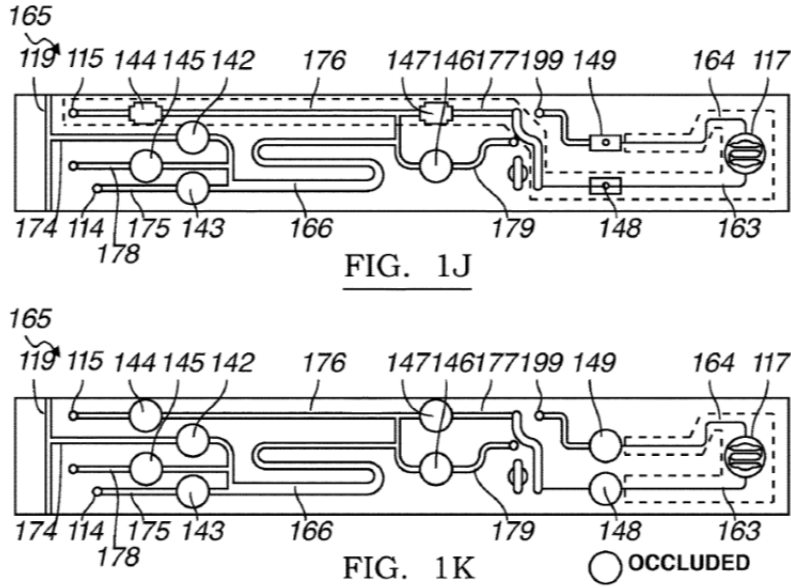
Claim	Claim Language	Infringement Evidence
		 <p>The diagram illustrates a microfluidic device. The top portion shows a top-down view of a chip with a grid of channels and valves. The bottom portion is a detailed cross-sectional view of a specific channel. Labels in the bottom view include: 'Inlet' (red), 'First Channel' (purple), 'Vent' (red), 'First valve' (red), 'Second valve' (red), 'Second Channel' (green), and 'Reaction Chamber' (red). The flow path is indicated by colored lines: purple for the first channel and green for the second channel.</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

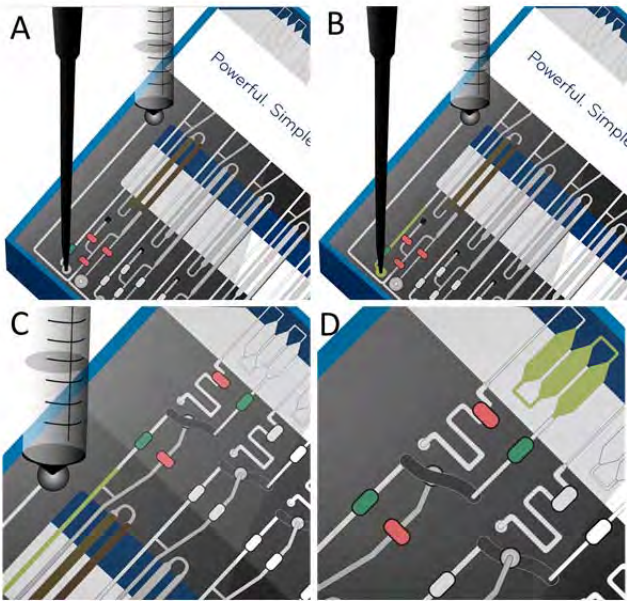
Claim	Claim Language	Infringement Evidence
		 <p>The diagram consists of four panels labeled A, B, C, and D, illustrating the operation of a microfluidic cartridge. Panels A and B show a pipette tip dispensing liquid into a well of the cartridge. Panels C and D show the internal fluidic pathways, including valves and PCR regions. A detailed inset below panels C and D shows a close-up of the internal components, with labels for 'Second valve', 'First valve', and 'PCR' regions. The text 'Powerful. Simple' is visible on the top right of panels A and B.</p> <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module,

Claim	Claim Language	Infringement Evidence
		<p>the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads.</p> <ul style="list-style-type: none"> • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber. <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve

Claim	Claim Language	Infringement Evidence
		<p>guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p>

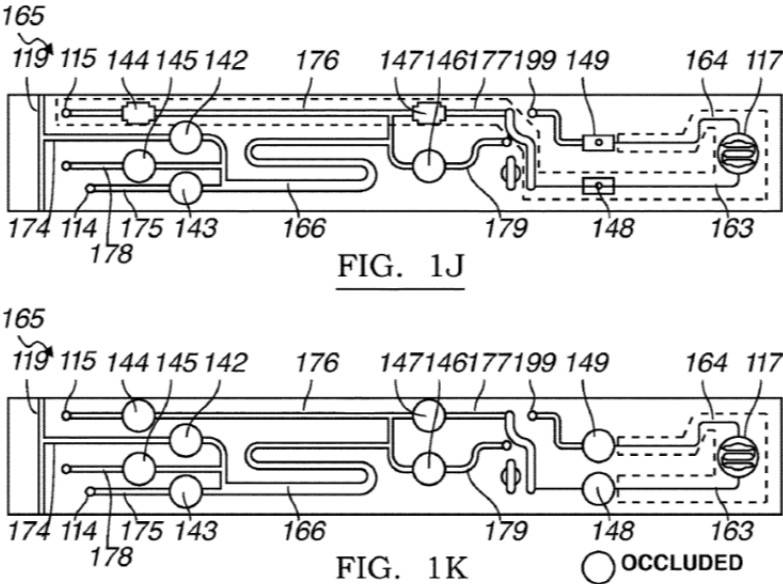
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”)at Figs. 1J and 1K:

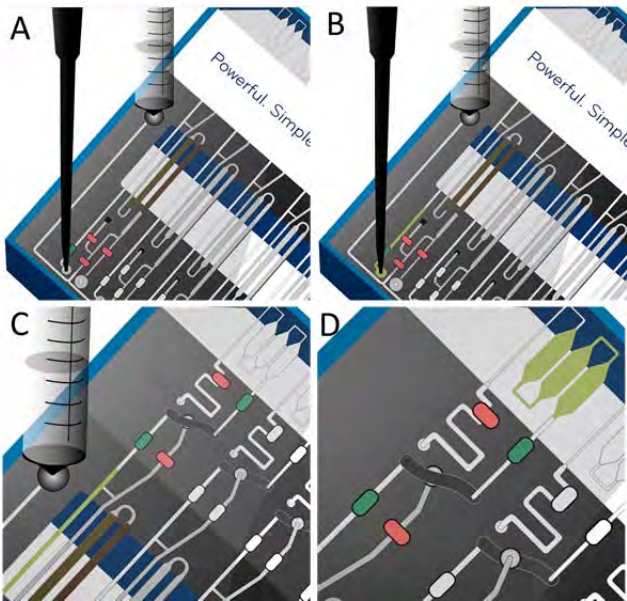
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1297 488 1430 521">FIG. 1J</p> <p data-bbox="1297 789 1430 821">FIG. 1K</p> <p data-bbox="1541 789 1703 821">○ OCCLUDED</p> <ul data-bbox="842 854 1892 1211" style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
10(e)	a first channel leading from the inlet, via the first valve, to a reaction chamber; and	The accused microfluidic substrate comprises a first channel leading from the inlet, via the first valve, to a reaction chamber.

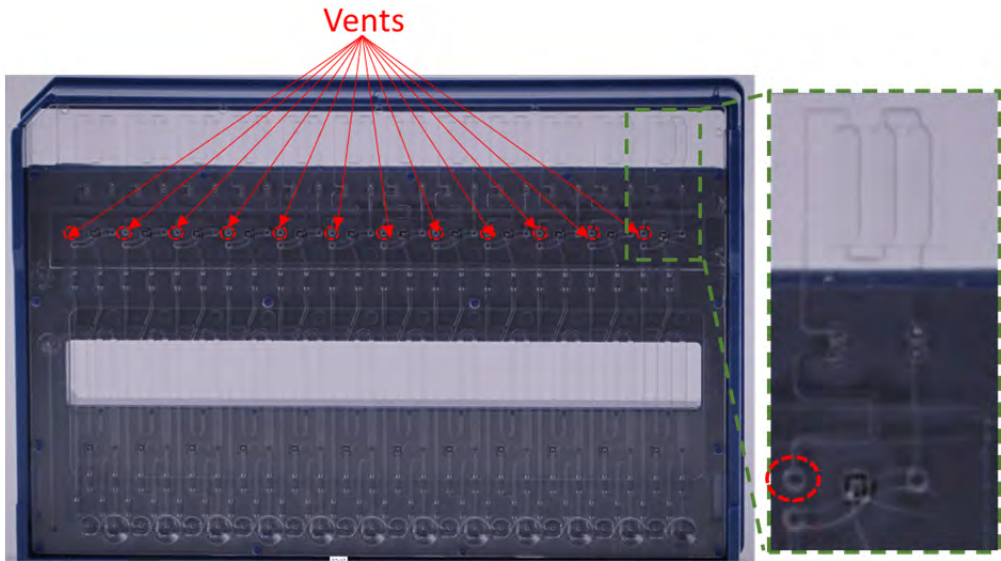
Claim	Claim Language	Infringement Evidence
		<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODx (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (<u>Exhibit 16</u>)</p> <ul style="list-style-type: none"> • “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction

Claim	Claim Language	Infringement Evidence
		<p>perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p> <ul style="list-style-type: none"> • US Patent No. 9,738,887 at 13:35-42 (“The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be

Claim	Claim Language	Infringement Evidence
		<p>eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/or detection.")</p> <ul style="list-style-type: none"> US Patent No. 9,738,887 at 15:31-35 ("The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117.") US Patent No. 9,738,887 at 17:27-49 ("Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.") US Patent No. 9,738,887 at Figs. 1J and 1K:

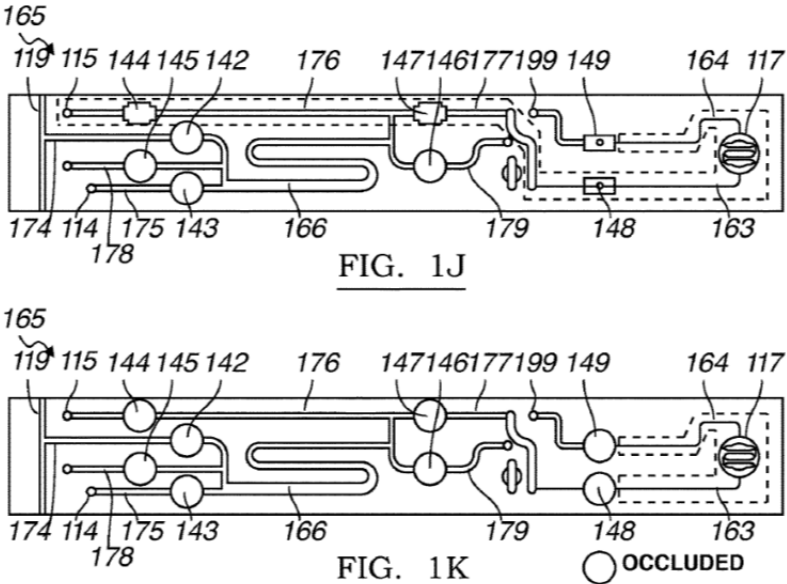
Claim	Claim Language	Infringement Evidence
		 <p>FIG. 1J</p> <p>FIG. 1K</p> <p>○ OCCLUDED</p>
10(f)	a second channel leading from the reaction chamber, via the second valve, to a vent,	<p>In the accused microfluidic substrate, each of the plurality of sample lanes comprises a microfluidic network having, in fluid communication with one another, a second channel leading from the reaction chamber, via the second valve, to a vent.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p>

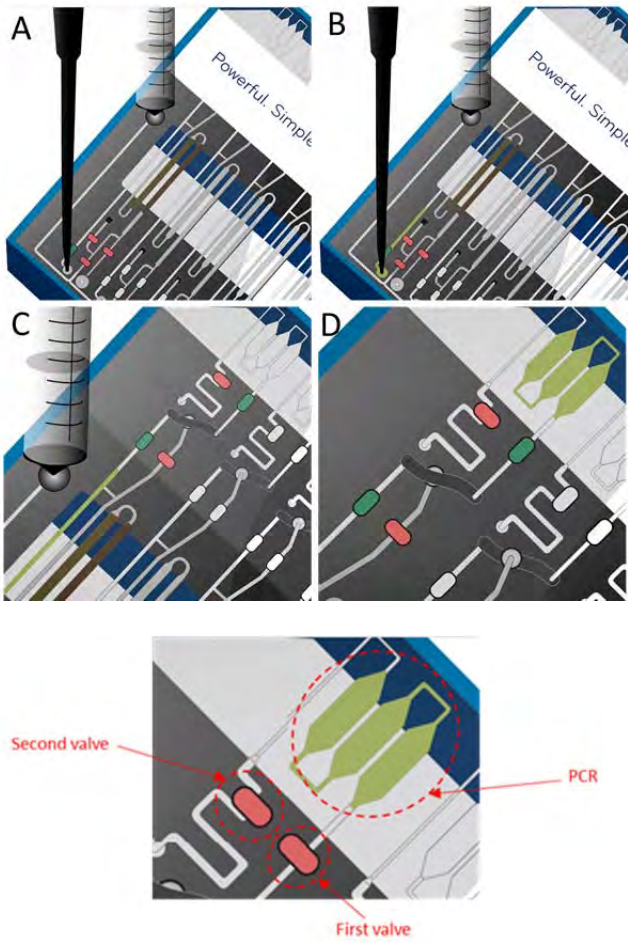
Claim	Claim Language	Infringement Evidence
		 <p data-bbox="842 873 1877 1019">On information and belief, in the accused microfluidic substrate, each of the plurality of sample lanes comprises a microfluidic network having, in fluid communication with one another, . . . a second channel leading from the reaction chamber, via the second valve, to a vent.</p> <ul data-bbox="842 1024 1020 1057" style="list-style-type: none"> • <i>Id.</i> at 2:10

Claim	Claim Language	Infringement Evidence
		 <p>The image shows a cartridge assembly with a series of vents along the top edge. Red arrows point from the word 'Vents' to these vents. A dashed green box highlights a specific component on the right side of the assembly, which is a circular feature with a red dashed circle around it.</p> <p>US9101930 (Exhibit 25)</p> <ul style="list-style-type: none"> Claim 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising: a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer; a first fluidic pathway, formed by at least a portion of the first layer; and a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, are each at least partially separated from the corrugated surface of the intermediate substrate by an elastomeric layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate. Claim 11. The cartridge of claim 10, wherein the first layer is a unitary

Claim	Claim Language	Infringement Evidence
		<p>construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.</p> <ul style="list-style-type: none"> • Claim 13. The cartridge of claim 11, further comprising a heating region as a recessed region of the first layer that is parallel to the set of parallel voids of the corrugated surface, and a vent region, such that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber. • Claim 15. The cartridge of claim 13, wherein at least of the first fluidic pathway and the second fluidic pathway is coupled to an end vent configured to provide fine metering of fluid flow. <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent

Claim	Claim Language	Infringement Evidence
		<p>port, the fluid port, and the detection chamber.</p> <ul style="list-style-type: none"> Claim 10. The cartridge of claim 1, wherein a terminal portion of the fluidic pathway is coupled to an end vent, configured to provide fine metering of fluid flow. U.S. Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) U.S. Patent No. 9,738,887 at Figs. 1J and 1K:

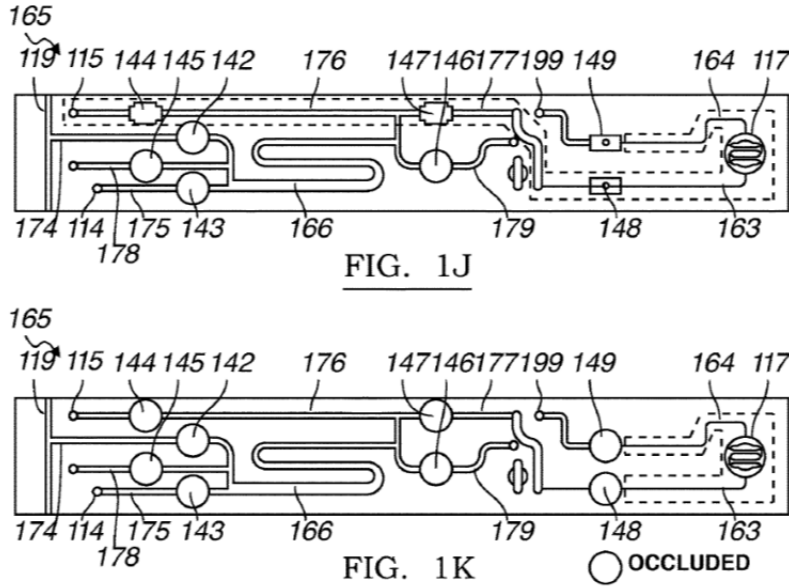
Claim	Claim Language	Infringement Evidence
		 <ul style="list-style-type: none"> U.S. Patent No. 8,738,887 at 15:4-6 (“A fluidic pathway 165 may also further comprise an end vent 199, which functions to prevent any fluid from escaping the microfluidic channel.”)
10(g)	wherein the first valve and the second valve are configured to isolate the reaction chamber from the inlet and the vent to prevent movement of fluid into or out of the reaction chamber,	<p>In the accused microfluidic substrate, the first valve and the second valve are configured to isolate the reaction chamber from the inlet and the vent to prevent movement of fluid into or out of the reaction chamber.</p> <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

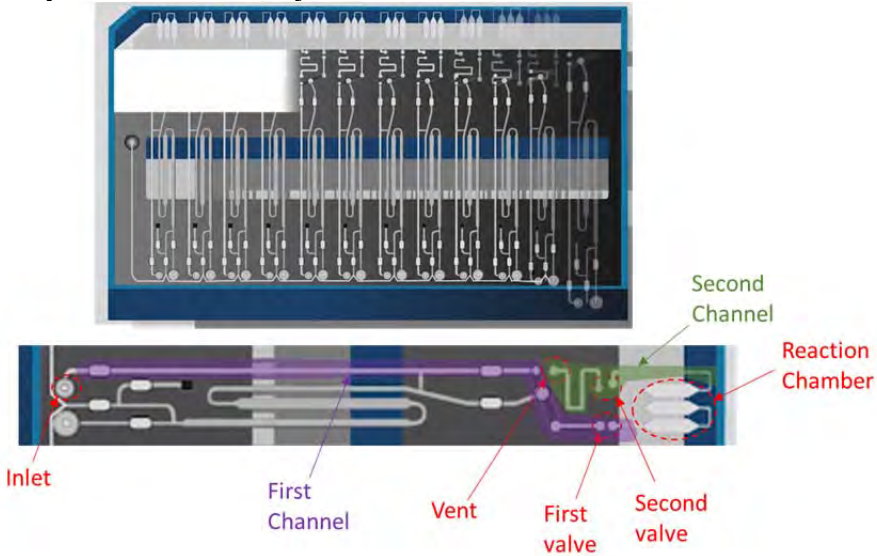
Claim	Claim Language	Infringement Evidence
		 <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module,

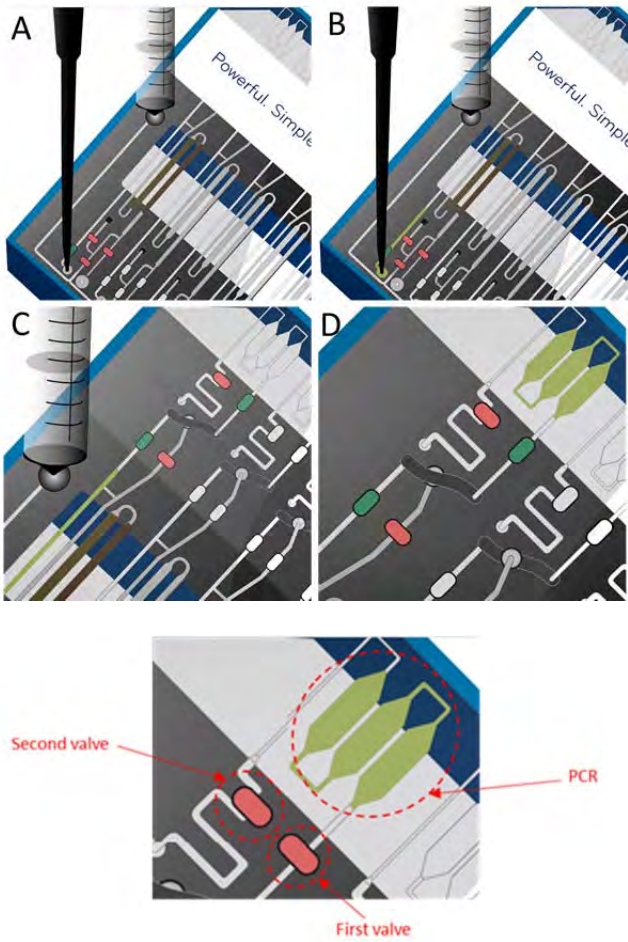
Claim	Claim Language	Infringement Evidence
		<p>the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads.</p> <ul style="list-style-type: none"> • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber. <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve

Claim	Claim Language	Infringement Evidence
		<p>guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p>

Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> <li data-bbox="842 237 1892 488">US Patent No. 9,738,887 at 12:11-19 (“When not in operation, however, the normally closed position 43 is configured to prevent leakage and/or fluid bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.”) <li data-bbox="842 496 1892 1333">US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) <li data-bbox="842 1341 1451 1365">US Patent No. 9,738,887 at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1297 488 1430 521">FIG. 1J</p> <p data-bbox="1297 789 1430 821">FIG. 1K</p> <p data-bbox="1541 789 1703 821">○ OCCLUDED</p> <ul data-bbox="842 854 1892 1211" style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
10(h)	wherein the first valve is spatially separated from the inlet and the second valve is spatially	In the accused microfluidic substrate, the first valve is spatially separated from the inlet and the second valve is spatially separated from the vent.

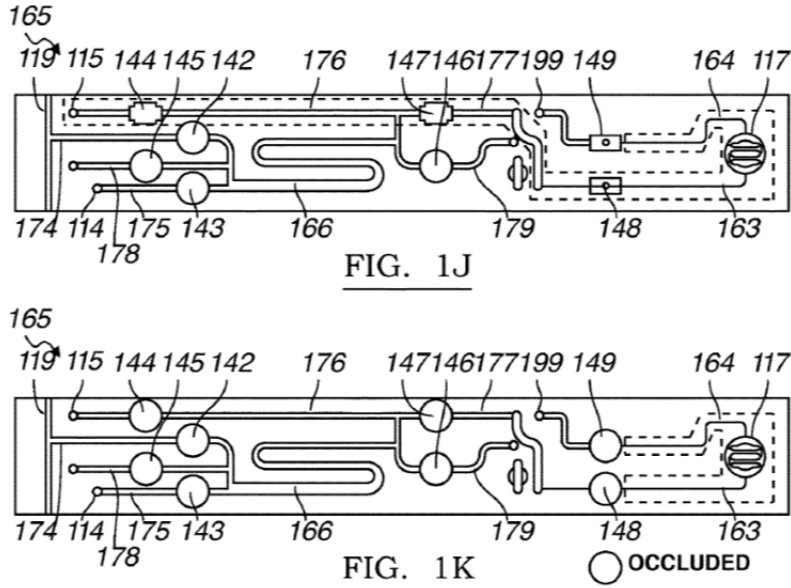
Claim	Claim Language	Infringement Evidence
	separated from the vent,	<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59  <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08

Claim	Claim Language	Infringement Evidence
		 <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> Claim 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of occlusion positions defined by an elastomeric layer of the cartridge, the method comprising: aligning the cartridge at a cartridge platform of a molecular diagnostic module,

Claim	Claim Language	Infringement Evidence
		<p>the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic pathway; capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field; and moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads.</p> <ul style="list-style-type: none"> • Claim 30. The method of claim 29, further comprising: delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids delivering the volume of nucleic acids through a reagent port coupled to the fluidic pathway; receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample; occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber. <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> • Claim 12. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer comprising a sample port and a detection chamber; an elastomeric layer; an intermediate substrate including a set of valve

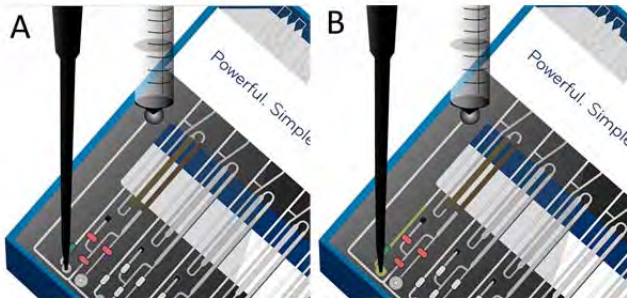
Claim	Claim Language	Infringement Evidence
		<p>guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position, wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation; wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.</p>

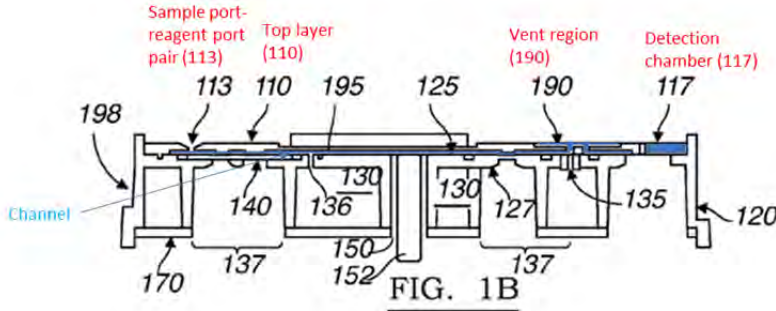
Claim	Claim Language	Infringement Evidence
		<ul style="list-style-type: none"> US Patent No. 9,738,887 at 17:27-49 (“Thereafter in the first embodiment, as shown in FIG. 11, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. -20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position [148] (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.”) US Patent No. 9,738,887 at at Figs. 1J and 1K:

Claim	Claim Language	Infringement Evidence
		 <p data-bbox="1297 488 1430 521">FIG. 1J</p> <p data-bbox="1297 789 1430 821">FIG. 1K</p> <p data-bbox="1541 789 1703 821">○ OCCLUDED</p> <ul data-bbox="842 854 1892 1219" style="list-style-type: none"> US Patent No. 9,738,887 at 16:4-25 (“In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.”)
10(i)	wherein the reaction chamber, the first channel, and the second channel are formed in a first side of the microfluidic substrate,	On information and belief, in the accused microfluidic substrate, the reaction chamber, the first channel, and the second channel are formed in a first side of the microfluidic substrate.


Claim	Claim Language	Infringement Evidence
		<p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent port, the fluid port, and the detection chamber. U.S. Patent No. 9,738,887 at Fig 1B <p>FIG. 1B</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 13:65-14:2. (“A fluidic pathway 165 of the set of fluidic pathways 160 may comprise portions (i.e. microfluidic channels) that are located on both sides of the top layer 110, but is preferably located primarily on the bottom side of the top layer (in the orientation shown in FIG. 1B).”) U.S. Patent No. 9,738,887 at 14:19-14:28. (“In one variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 11B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a vent region 190 on the top side of the top

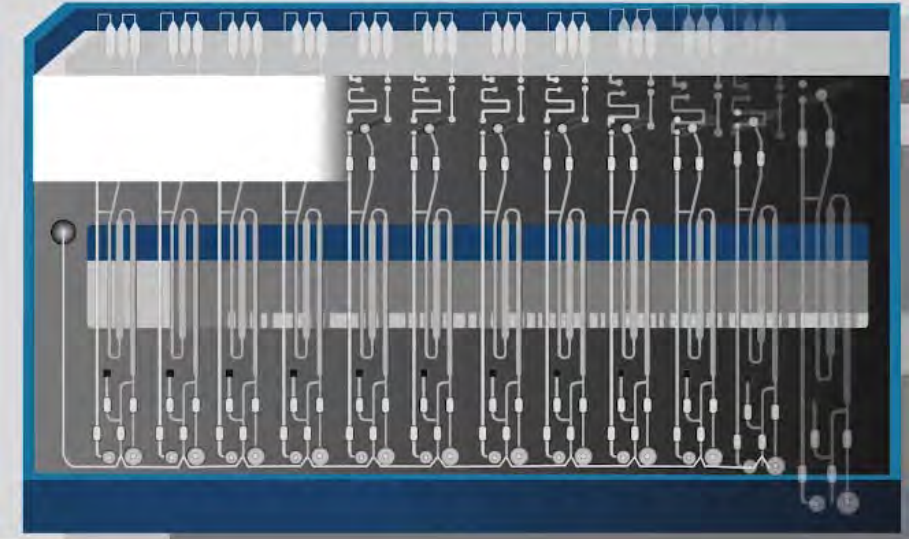
Claim	Claim Language	Infringement Evidence
		<p>layer 110. All other segments of the fluidic pathway 165 are preferably located on the bottom side of the top layer 110, allowing the fluidic pathway 165 to be sealed by the film layer 125 without requiring a separate film layer to seal channels located on the top of the top layer 110.”)</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,738,887 at 2:37-49. (“As shown in FIGS. 1A-1C, an embodiment of a microfluidic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample port-reagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; and a set of fluidic pathways 160, each formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140.”) • U.S. Patent No. 9,738,887 at 3:26-31. (“As shown in FIGS. 1B and 1C, the top layer 110 preferably comprises a set of sample port-reagent port pairs 112, a fluid port 118, a vent region 190, a heating region 195 crossing a capture segment 166 of a fluidic pathway 165, and a set of detection chambers 116.”) • U.S. Patent No. 9,738,887 at 5:66-6:17 (“In a first variation, as shown in FIGS. 1A and 11B, each detection chamber 117 in the set of detection chambers comprises a serpentine-shaped channel 16 for facilitating analysis of a solution of nucleic acids mixed with reagents... In a specific example of the first variation, each serpentine-shaped channel 16 is injected molded into the top layer 110 of the microfluidic cartridge 100, and the three interconnected portions of the serpentine-shaped channel 16 are each 1600 μm wide by 400 μm deep.”)
10(j)	wherein the inlet and the vent are formed in a second side of	On information and belief, in the accused microfluidic substrate, the inlet and the vent are formed in a second side of the microfluidic substrate opposite the first side

Claim	Claim Language	Infringement Evidence
	the microfluidic substrate opposite the first side, and	<p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> “A series of microfluidic valves guides the PCR-ready solution through the cartridge into three thin PCR chambers and the amplification process begins.” <i>Id.</i> at 3:58-4:08  <p>US9738887 (Exhibit 31)</p> <ul style="list-style-type: none"> Claim 1. A cartridge, configured to facilitate processing and detecting of a nucleic acid, comprising: a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber; an elastomeric layer; an intermediate substrate coupled to the first layer, such that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent port, the fluid port, and the detection chamber. Claim 2. The cartridge of claim 1 wherein the fluidic pathway is formed by at

Claim	Claim Language	Infringement Evidence
		<p>least a portion of the first layer and a portion of the elastomeric layer, is configured to be occluded upon manipulation of the elastomeric layer through the set of openings of the corrugated surface, and is configured to transfer a waste fluid to the chamber.</p> <ul style="list-style-type: none"> Claim 4. The cartridge of claim 2, wherein the chamber of the corrugated surface includes a waste inlet coupled to the fluidic pathway and a waste vent situated at a first side of the fluidic pathway, and wherein the cartridge further comprises a vent region directly opposed to the waste vent at a second side of the fluidic pathway. U.S. Patent No. 9,738,887 at Fig 1B  <p>FIG. 1B</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at Abstract (“A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste

Claim	Claim Language	Infringement Evidence
		<p>chamber, and to pass through the vent region”)</p> <ul style="list-style-type: none"> U.S. Patent No. 9,738,887 at 14:19-14:28. (“In one variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 11B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a vent region 190 on the top side of the top layer 110. All other segments of the fluidic pathway 165 are preferably located on the bottom side of the top layer 110, allowing the fluidic pathway 165 to be sealed by the film layer 125 without requiring a separate film layer to seal channels located on the top of the top layer 110.”) U.S. Patent No. 9,738,887 at 14:35-42. (“In this variation, the fluidic pathway 165 thus crosses the thickness of the top layer 110 upstream of the first segment running to the detection chamber 163, and crosses the thickness of the top layer 110 downstream of the segment running away from the detection chamber 164, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110.”) U.S. Patent No. 9,738,887 at 23:52-60 (“The injection molding process also defines the shared fluid port 118 of the top layer 110, and the vent region 190, which is recessed 0.5 mm into the top surface of the top layer 110 (in the orientation shown in FIG. 11B), and is covered with a polytetrafluoroethylene membrane, which is hydrophobic, gas permeable, and liquid impermeable. A paper label is bonded with adhesive to the top layer 110 over the vent region 190, which serves to identify the cartridge and protect the vent region 190, as shown in FIGS. 11A and 11B.”)
10(k)	wherein the first valve in each of the plurality of sample lanes is operated independently of any other first valve.	<p>In the accused microfluidic substrate, the first valve in each of the plurality of sample lanes is operated independently of any other first valve.</p> <p><i>NeuMoDx_Quant_HCV_CVS_2018.pdf (Exhibit 18)</i></p> <ul style="list-style-type: none"> Describing “...microfluidic cartridges capable of performing independent sample processing and real-time PCR.”

Claim	Claim Language	Infringement Evidence
		 <p><i>NeuMoDx Molecular N96 and N288 Overview and Animation</i>, NEUMODX (Nov. 6, 2018, 3:50 PM), http://www.neumodx.com/our-solutions/ - linking to “VIDEO NeuMoDx™ WORKFLOW” hyperlink at https://player.vimeo.com/video/299307936. (Exhibit 16)</p> <ul style="list-style-type: none"> • “This is the NeuMoDx microfluidic cartridge. Each microfluidic cartridge contains 12 independent lanes which allows for processing of up to 12 samples simultaneously.” <i>Id.</i> at 1:49-1:59

Claim	Claim Language	Infringement Evidence
		 <p data-bbox="793 816 1877 889"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/, last visited May 31, 2019 (Exhibit 10)</p> <ul data-bbox="846 898 1919 1222" style="list-style-type: none"> • “NeuMoDx™ Molecular Systems provide the industry’s first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays.” <p data-bbox="793 1255 1911 1328"><i>NeuMoDx™ Molecular Systems</i>, NEUMODX, http://www.neumodx.com/our-solutions/, last visited May 31, 2019 (Exhibit 11)</p> <ul data-bbox="846 1336 1877 1403" style="list-style-type: none"> • “NeuMoDx™ MOLECULAR SYSTEMS REVOLUTIONARY MOLECULAR DIAGNOSTIC SOLUTION Our patented, “sample to result”

Claim	Claim Language	Infringement Evidence
		<p>platform offers market-leading ease of use, true continuous random-access and rapid turnaround time while achieving [sic] optimal operational and clinical performance for our customers and their patients.”</p> <ul style="list-style-type: none"> • “The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from “sample to result”. The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents. <p>40600094_D-IFU-NeuMoDx-Cartridge-US-ONLY.pdf (Exhibit 19)</p> <ul style="list-style-type: none"> • “NeuMoDx™ Cartridge INSTRUCTIONS FOR USE... Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System... The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR.” <p>US9339812 (Exhibit 26)</p> <ul style="list-style-type: none"> • Claim 15. A method for processing and detecting nucleic acids from a set of biological samples with a cartridge having a set of fluidic pathways defined by an elastomeric layer, the method comprising: combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of nucleic acid-magnetic bead samples; aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set

Claim	Claim Language	Infringement Evidence
		<p>of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots; transferring substantially all of each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways; moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude at least one fluidic pathway of the set of fluidic pathways at a subset of occlusion positions for controlling a flow through the fluidic pathway; and detecting nucleic acids using a set of detection chambers coupled to the set of fluidic pathways.</p> <ul style="list-style-type: none"> • U.S. Patent No. 9,339,812 at 10:63-65 (“The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing.”)

EXHIBIT 40

(10) **Patent No.:** US 10,625,261 B2
(45) **Date of Patent:** *Apr. 21, 2020

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3/5027; B01L 3/52; B01L 7/52;
(Continued)

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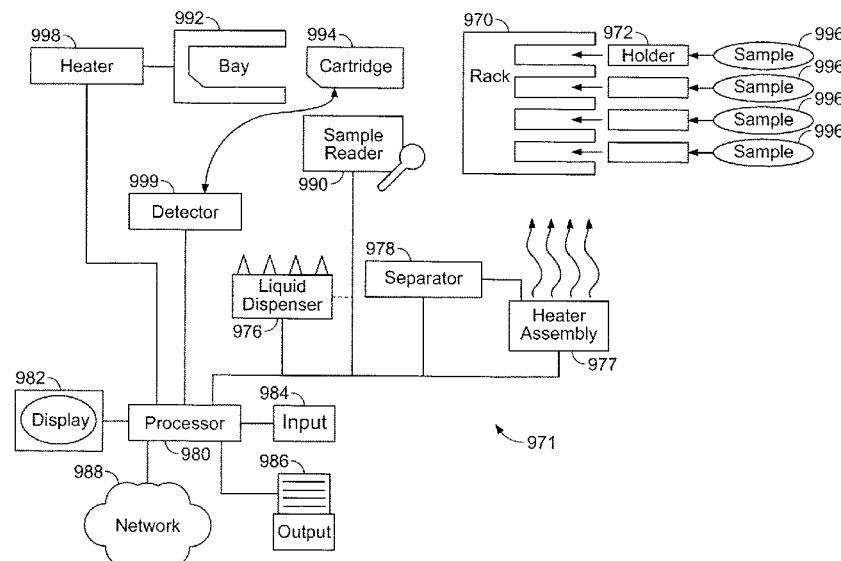
Related U.S. Application Data

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& Bear LLP

(57) **ABSTRACT**

The technology described herein generally relates to systems for extracting polynucleotides from multiple samples, particularly from biological samples, and additionally to systems that subsequently amplify and detect the extracted polynucleotides. The technology more particularly relates to microfluidic systems that carry out PCR on multiple samples of nucleotides of interest within microfluidic channels, and detect those nucleotides.

(52) **U.S. Cl.**
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(2013.01); ***B01L 3/5027*** (2013.01);
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Related U.S. Application Data

- No. 14/941,087, filed on Nov. 13, 2015, now Pat. No. 10,071,376, which is a continuation of application No. 12/218,498, filed on Jul. 14, 2008, now Pat. No. 9,186,677, which is a continuation-in-part of application No. 11/985,577, filed on Nov. 14, 2007, now Pat. No. 7,998,708.
- (60) Provisional application No. 60/959,437, filed on Jul. 13, 2007.
- (51) **Int. Cl.**
B01L 9/00 (2006.01)
F16K 99/00 (2006.01)
B01L 3/02 (2006.01)
B01L 9/06 (2006.01)
G01N 35/02 (2006.01)
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G01N 35/04 (2006.01)
- (52) **U.S. Cl.**
CPC **B01L 3/52** (2013.01);
B01L 7/52 (2013.01); **B01L 9/06** (2013.01);
B01L 9/527 (2013.01); **F16K 99/0001**
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99/0032 (2013.01); **F16K 99/0044** (2013.01);
F16K 99/0061 (2013.01); **B01L 2200/027**
(2013.01); **B01L 2200/10** (2013.01); **B01L**
2200/147 (2013.01); **B01L 2200/148**
(2013.01); **B01L 2200/16** (2013.01); **B01L**
2300/021 (2013.01); **B01L 2300/045**
(2013.01); **B01L 2300/06** (2013.01); **B01L**
2300/0627 (2013.01); **B01L 2300/0681**
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2300/0816 (2013.01); **B01L 2300/0832**
(2013.01); **B01L 2300/0867** (2013.01); **B01L**
2300/0887 (2013.01); **B01L 2300/18**
(2013.01); **B01L 2300/1822** (2013.01); **B01L**
2300/1827 (2013.01); **B01L 2300/1861**
(2013.01); **B01L 2400/0442** (2013.01); **B01L**
2400/0481 (2013.01); **B01L 2400/0487**
(2013.01); **B01L 2400/0611** (2013.01); **B01L**
2400/0677 (2013.01); **B01L 2400/0683**
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(2013.01); **G01N 2035/0425** (2013.01); **G01N**
2035/0436 (2013.01)
- (58) **Field of Classification Search**
CPC **B01L 9/06**; **B01L 9/527**; **F16K 99/0001**;
F16K 99/003; **F16K 99/0032**; **F16K**
99/0044; **F16K 99/0061**
See application file for complete search history.
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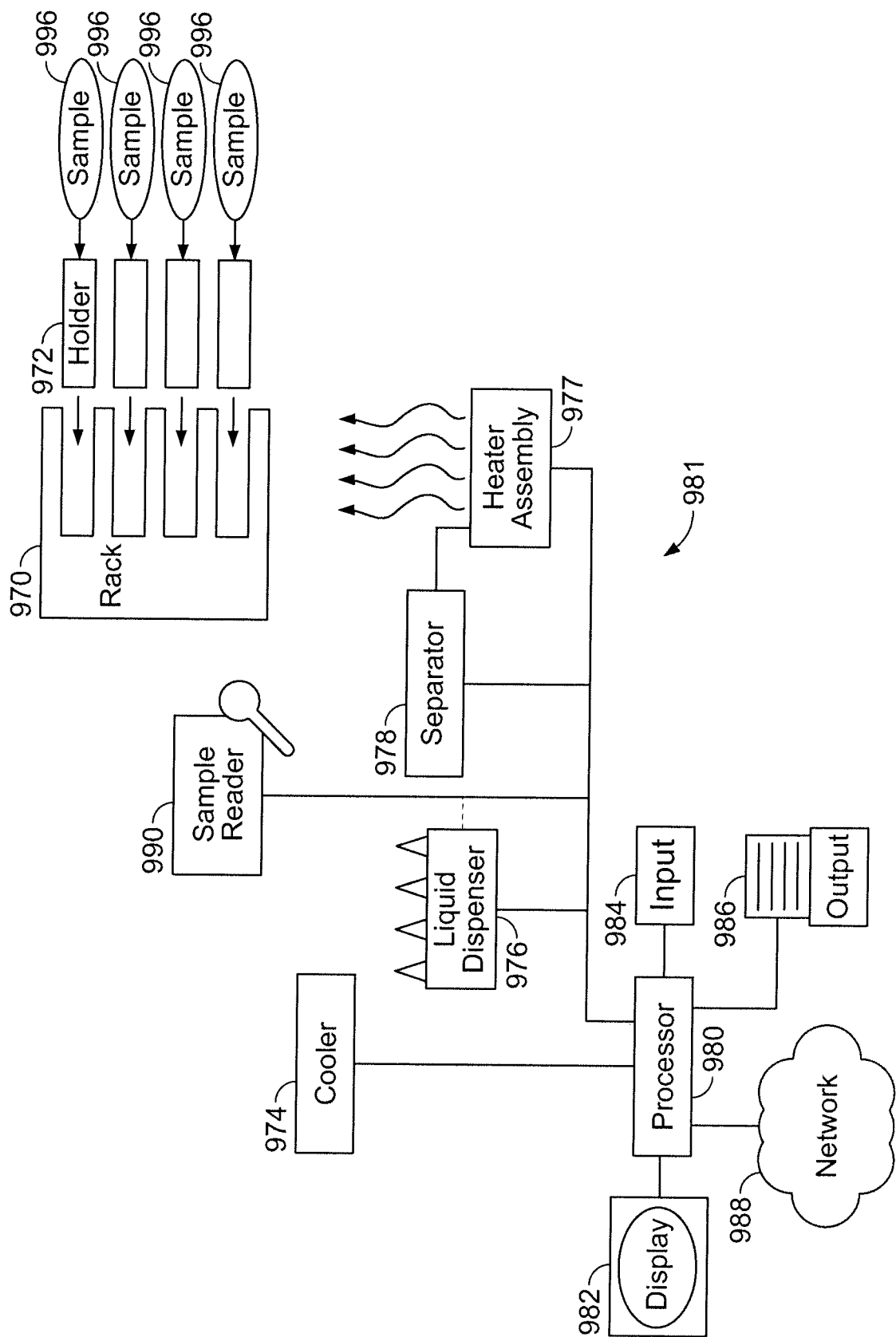


FIG. 1A

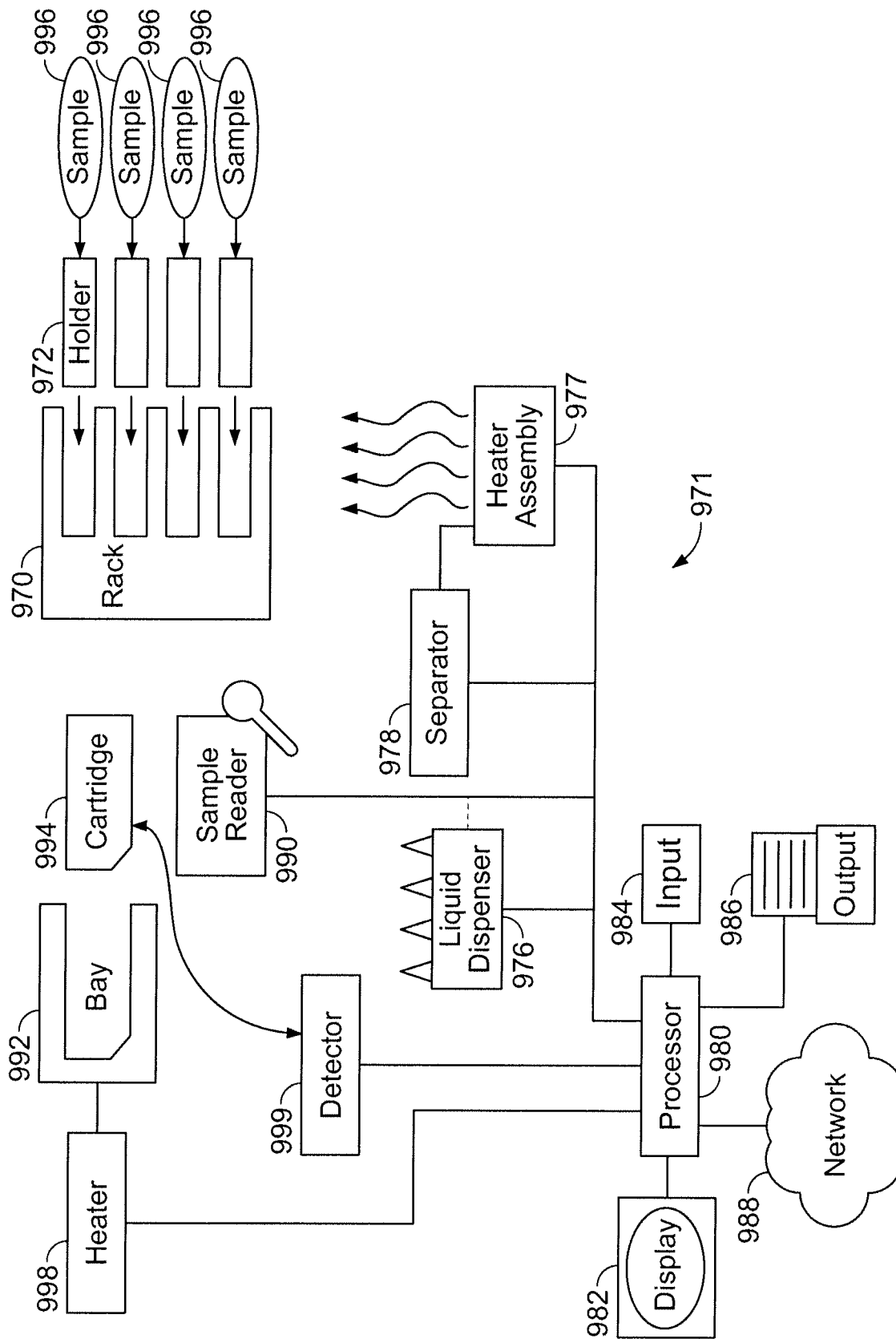


FIG. 1B

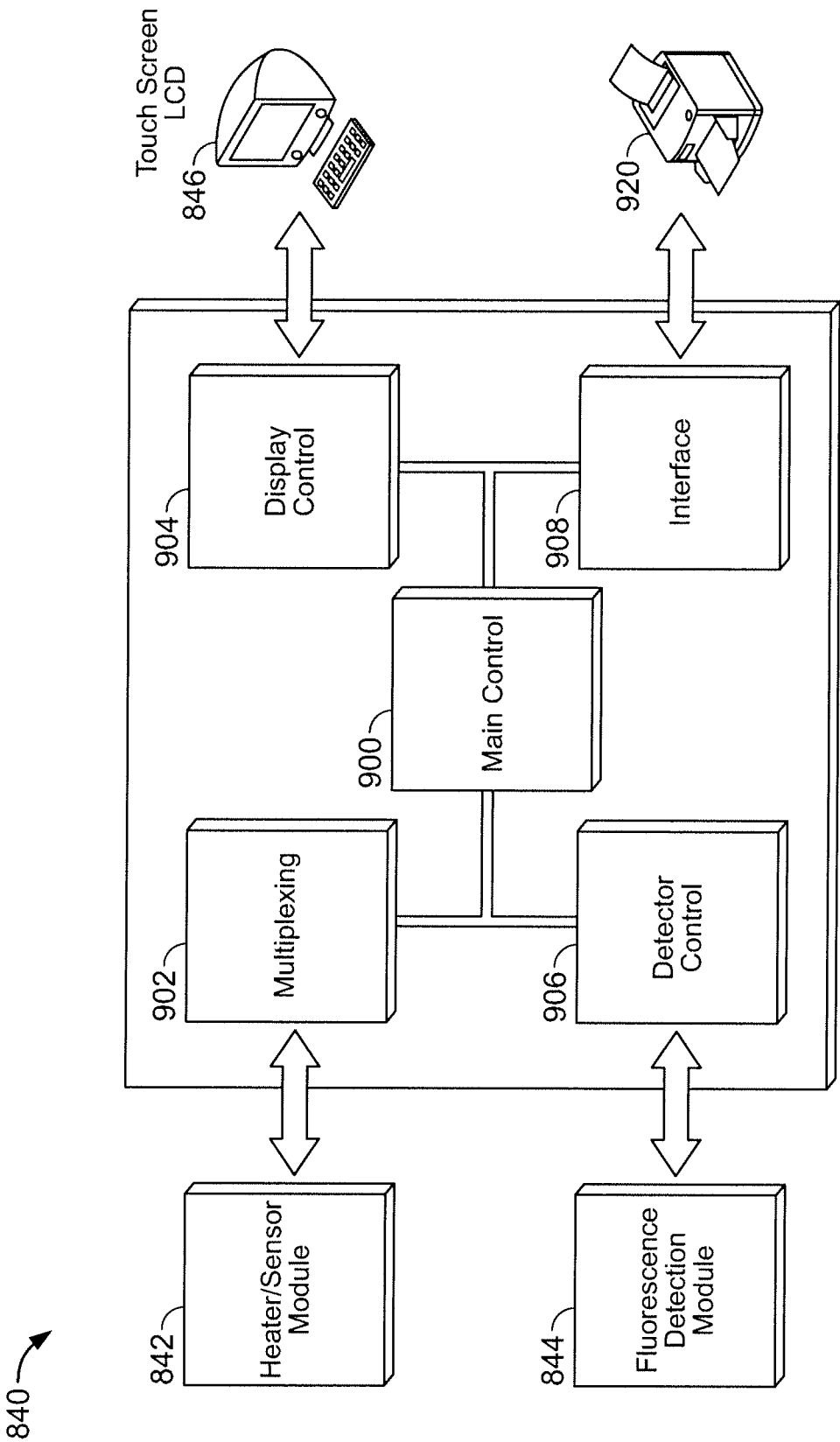


FIG. 2

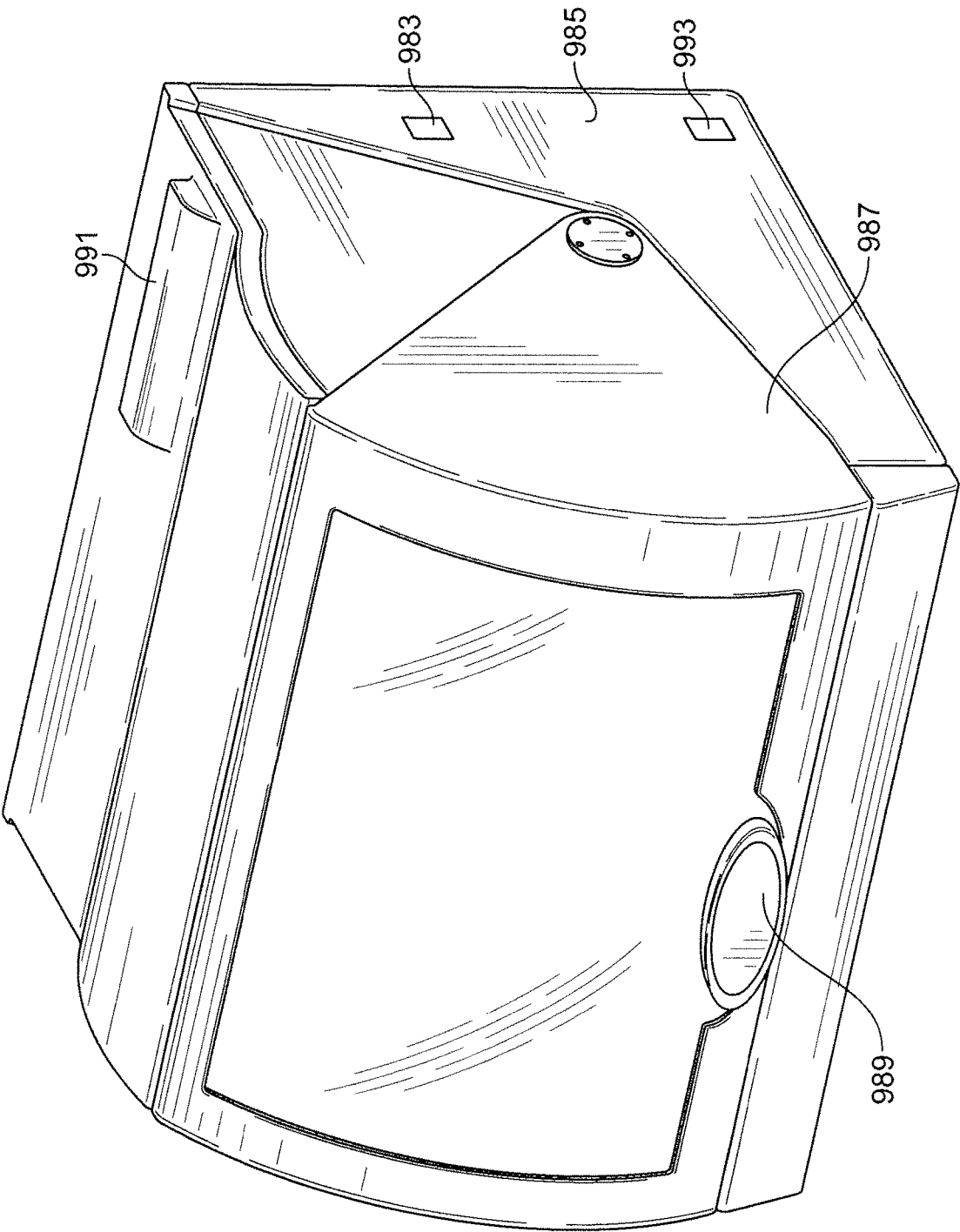


FIG. 3A

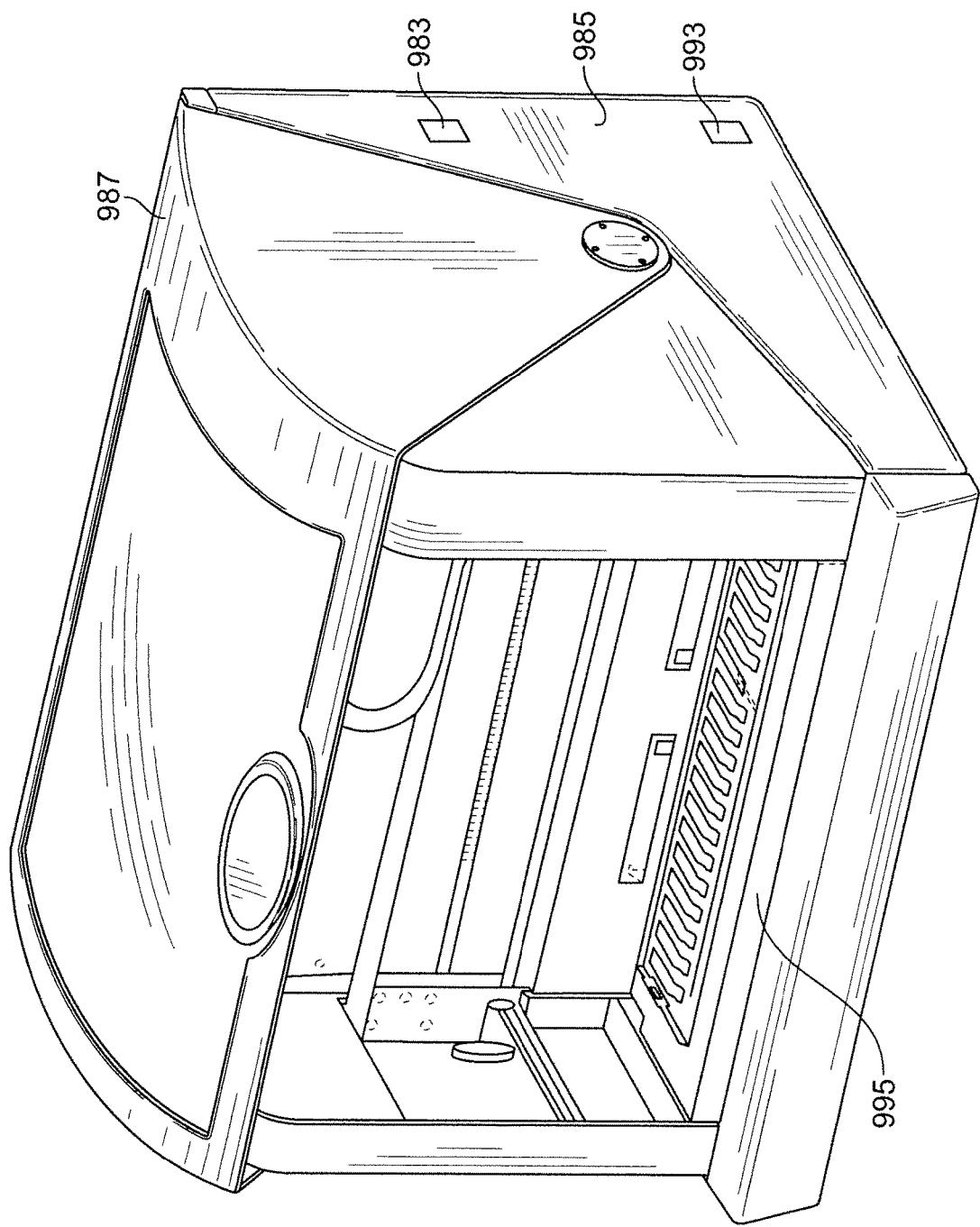
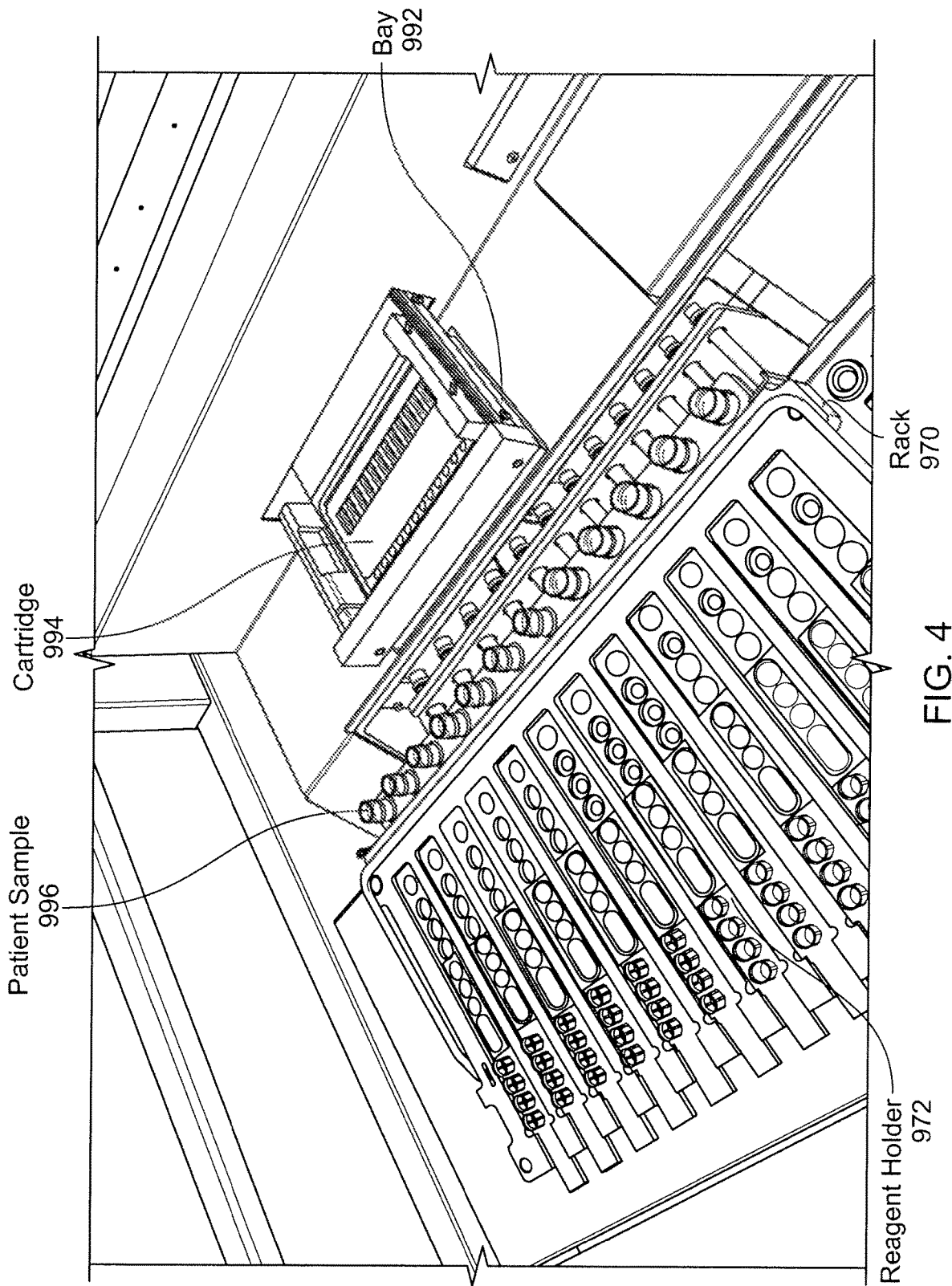
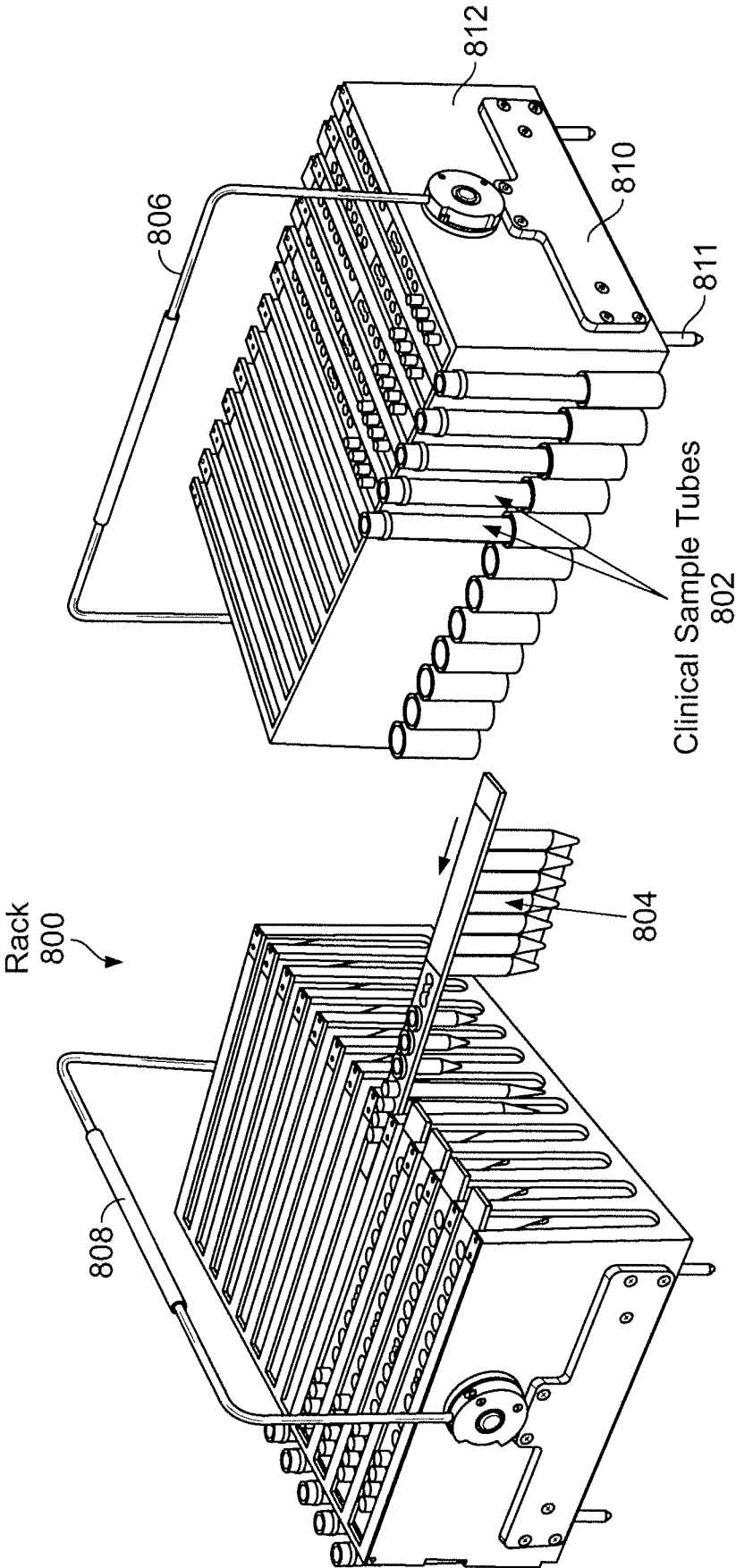


FIG. 3B





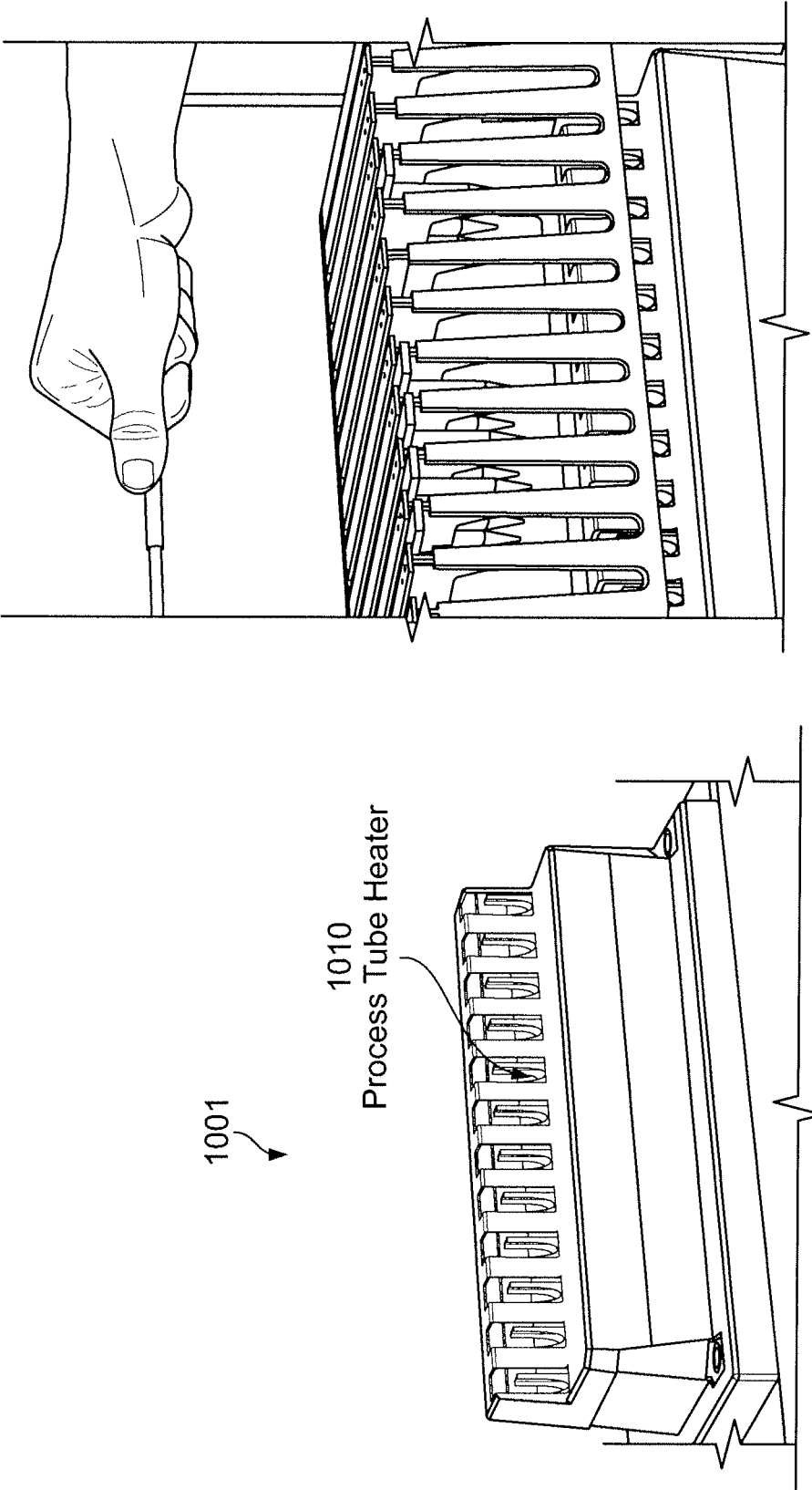
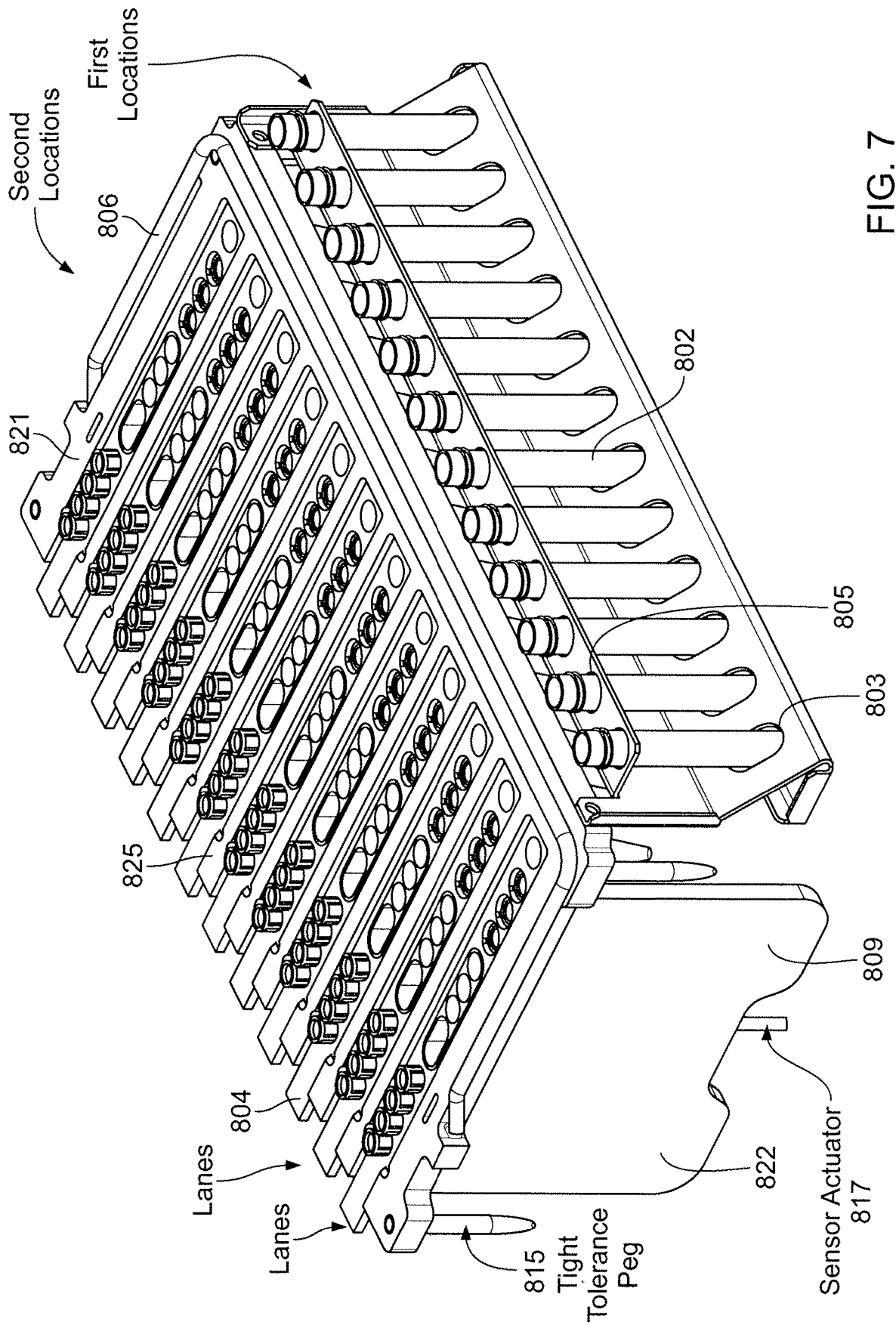


FIG. 6



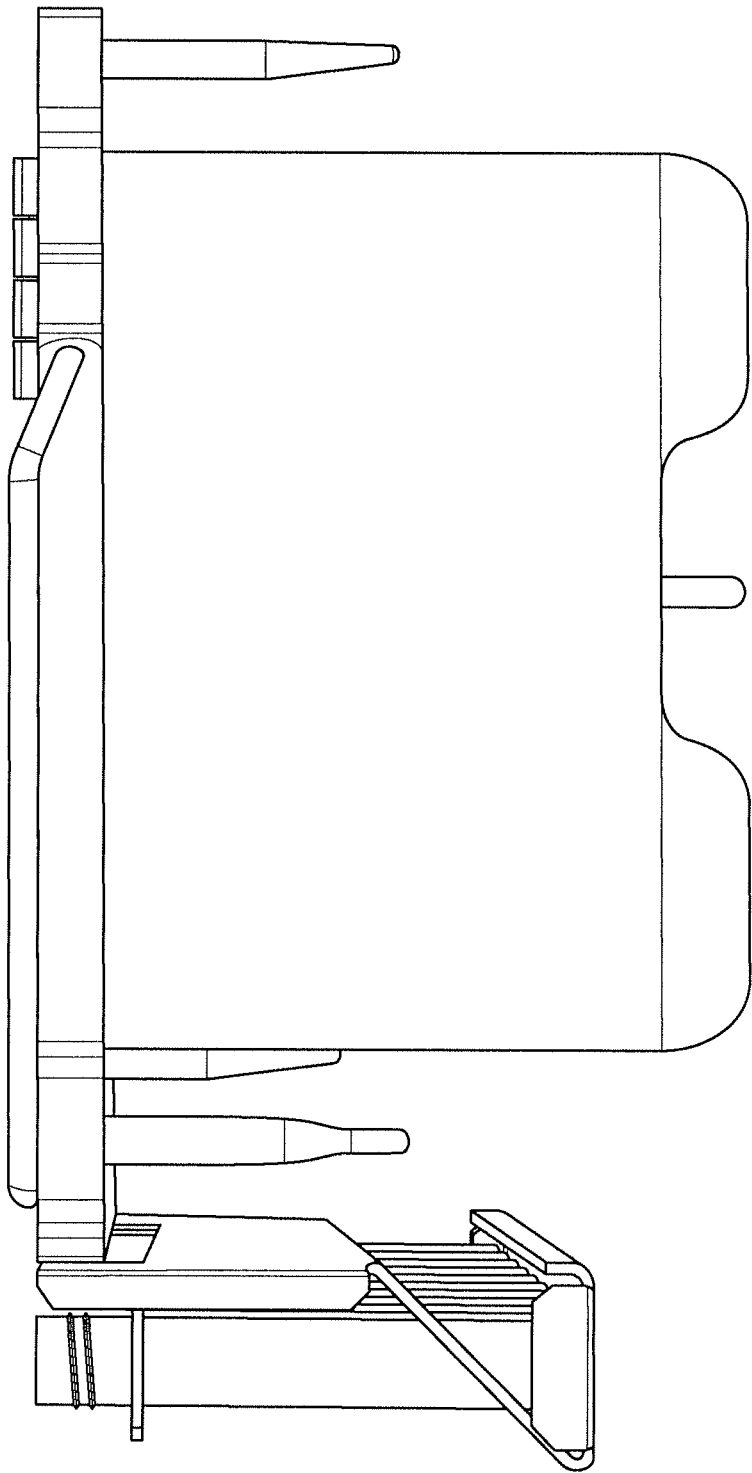


FIG. 8A

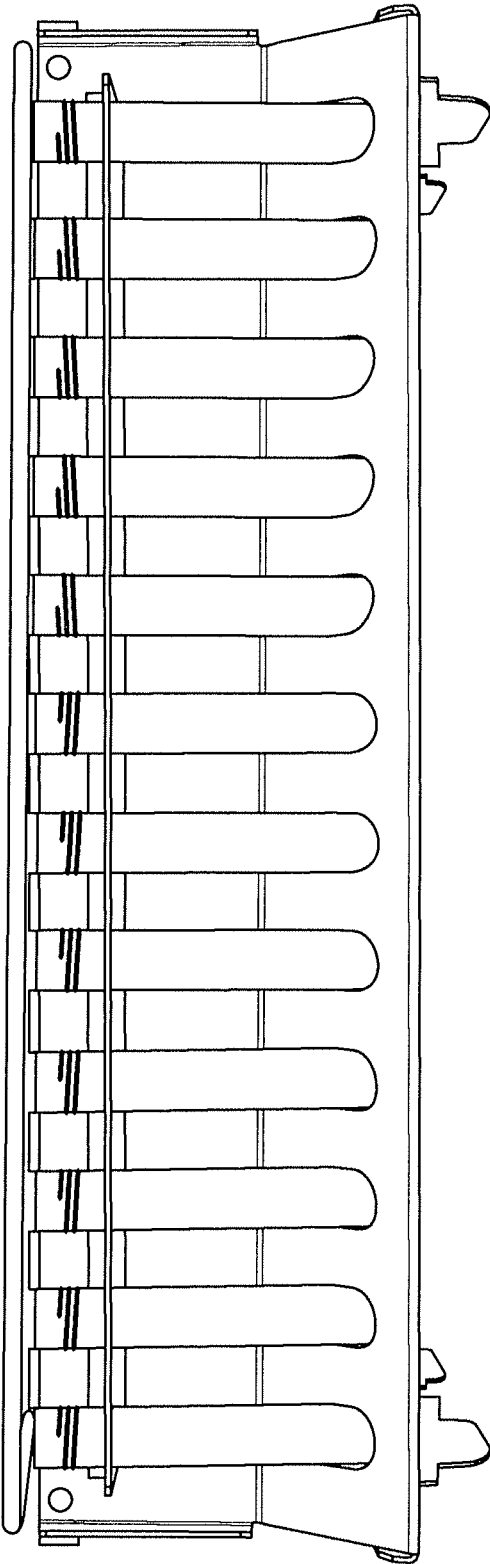


FIG. 8B

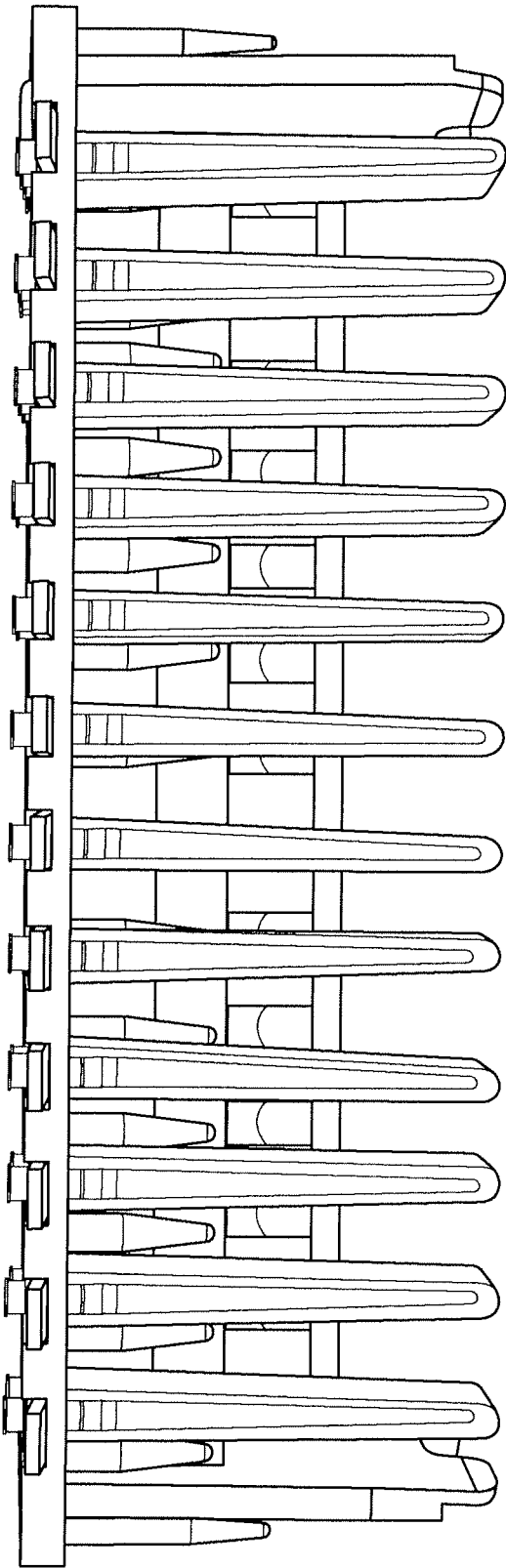


FIG. 8C

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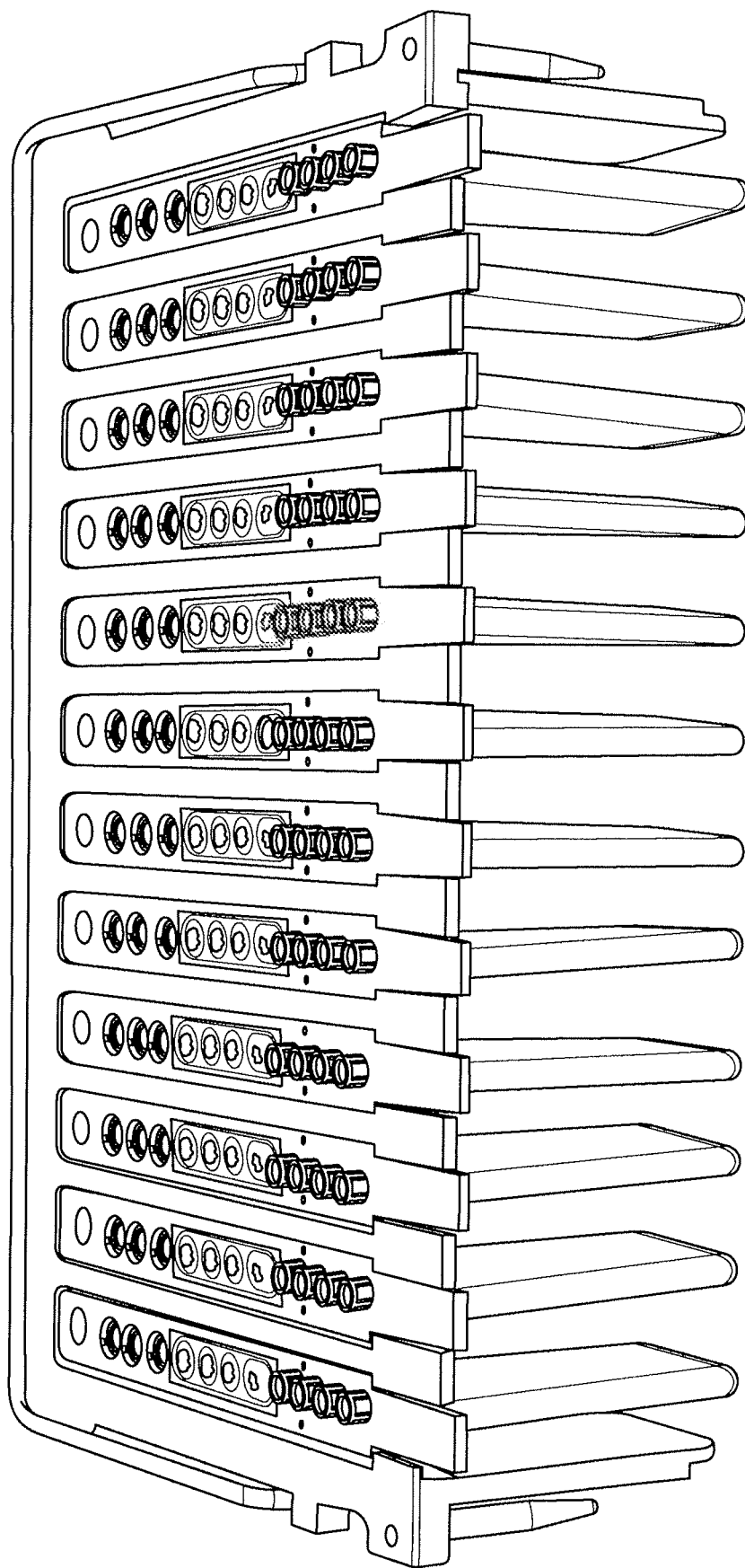


FIG. 8D

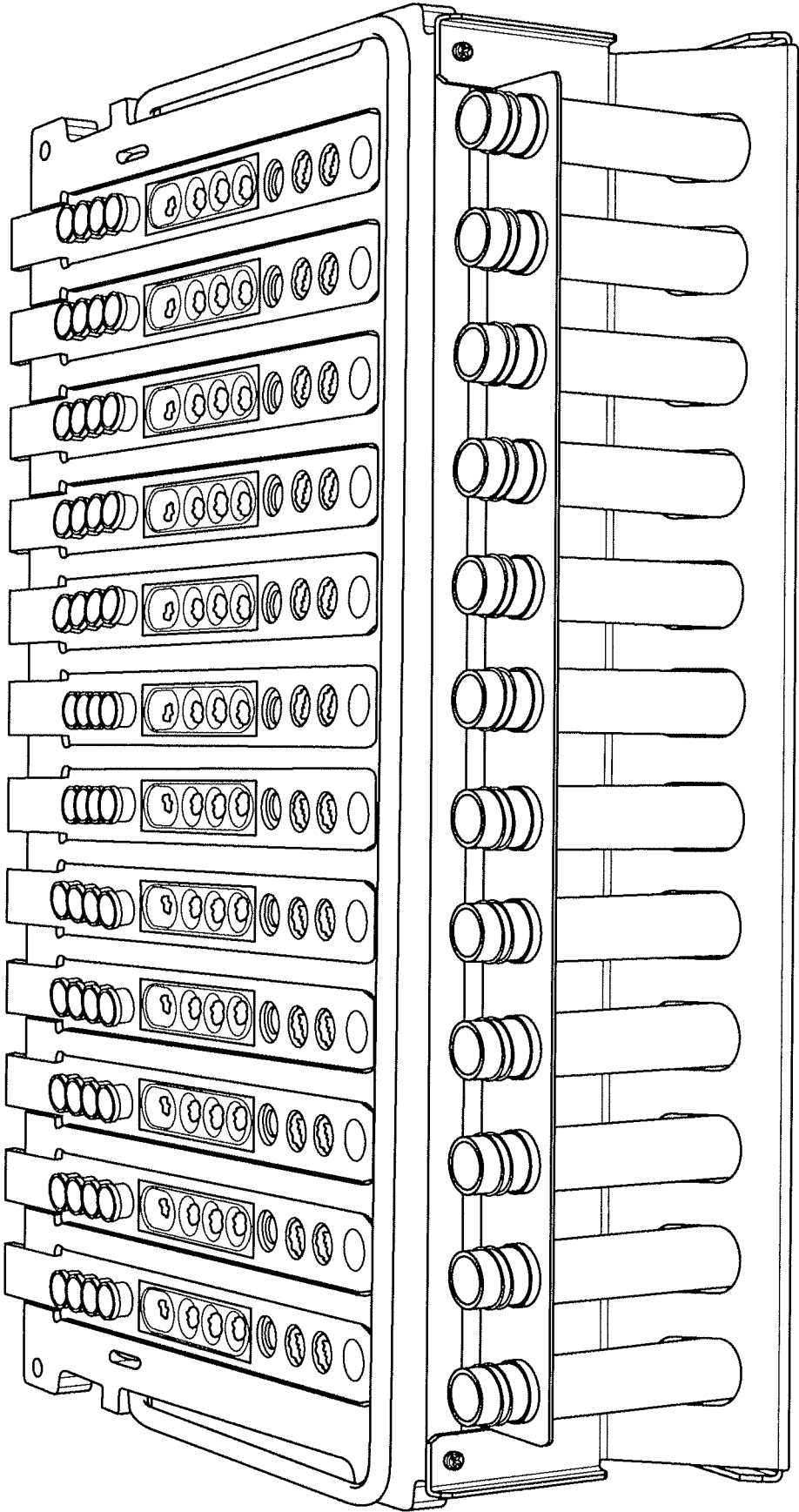


FIG. 8E

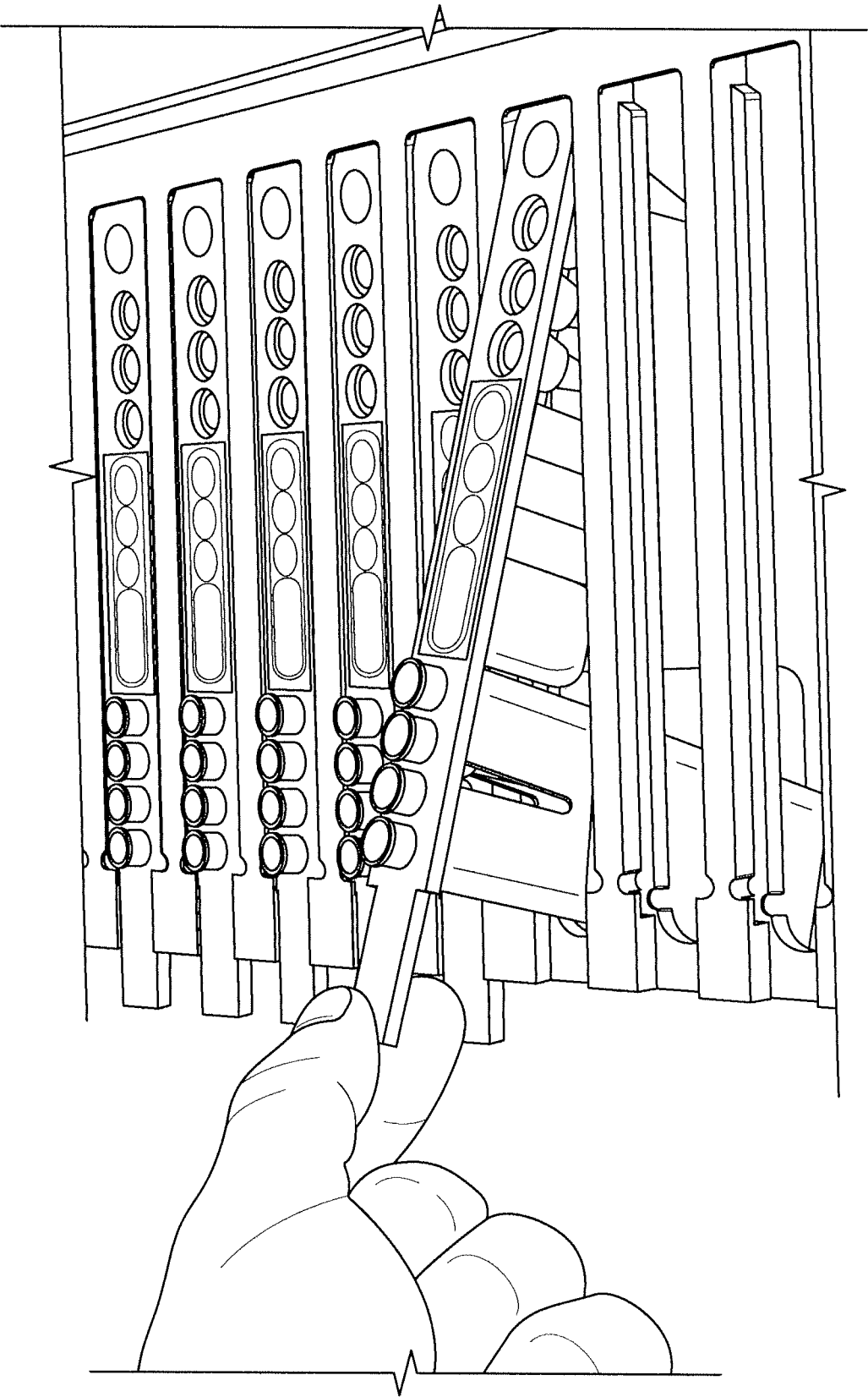


FIG. 8F

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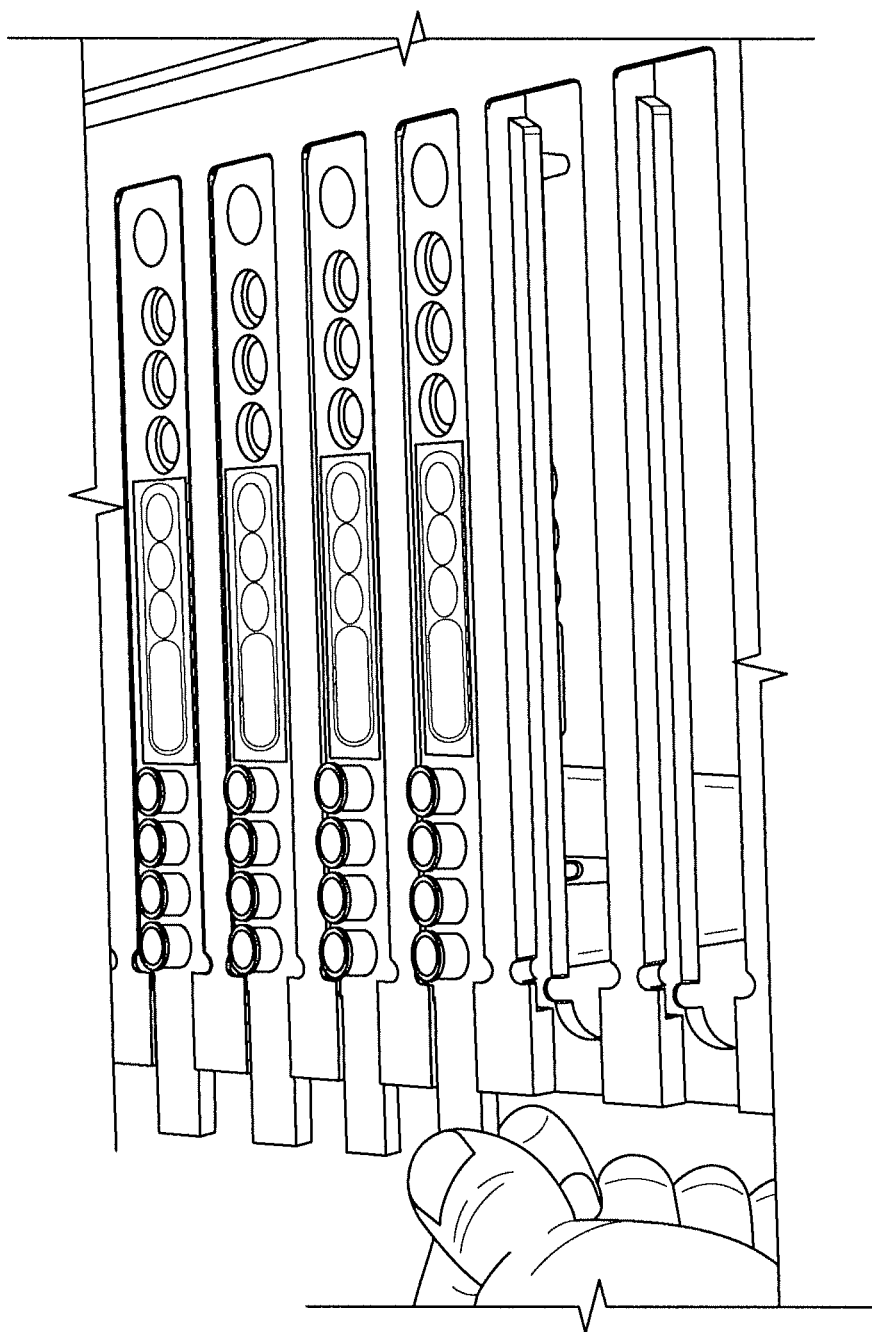


FIG. 8G

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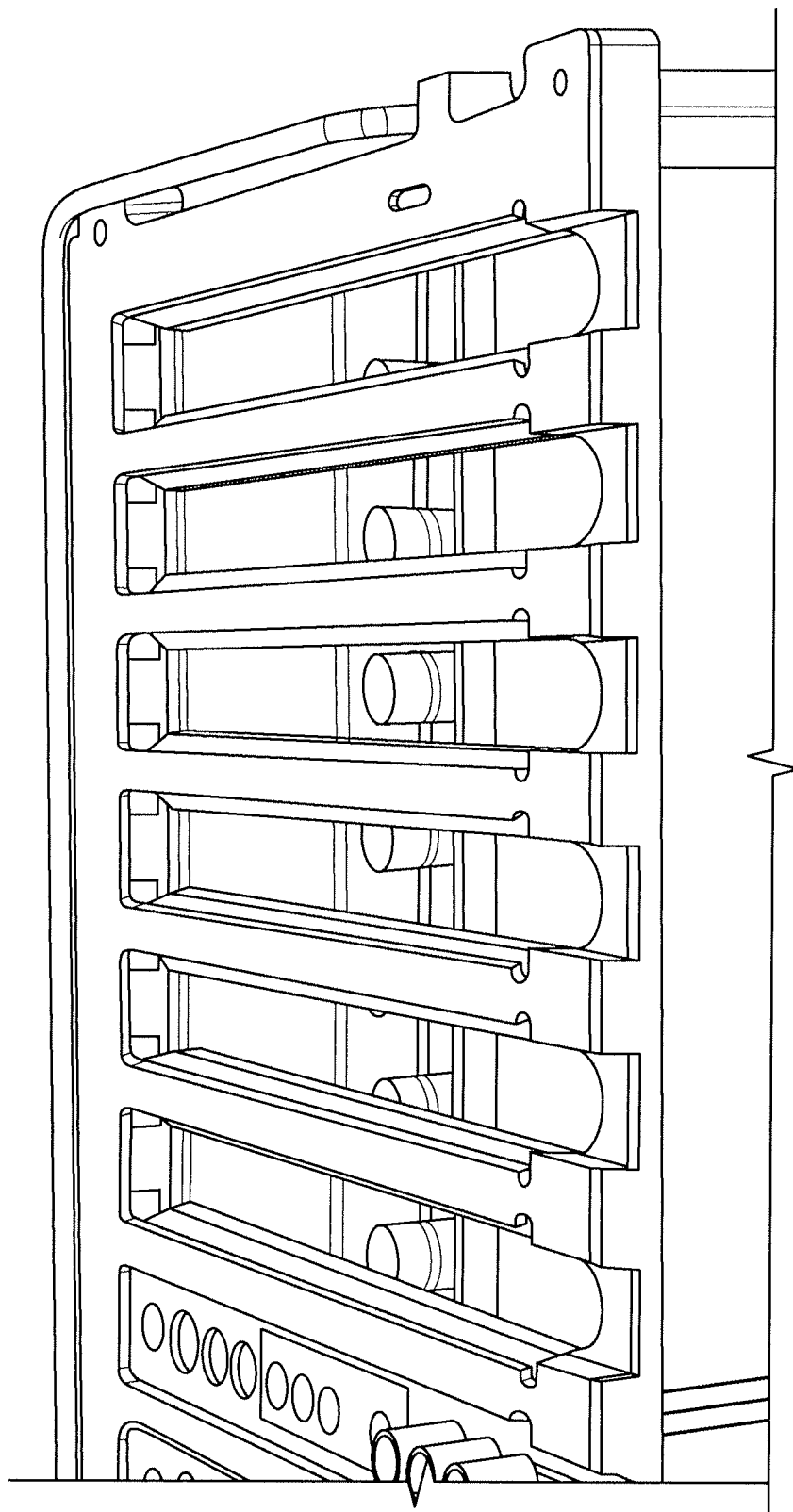


FIG. 8H

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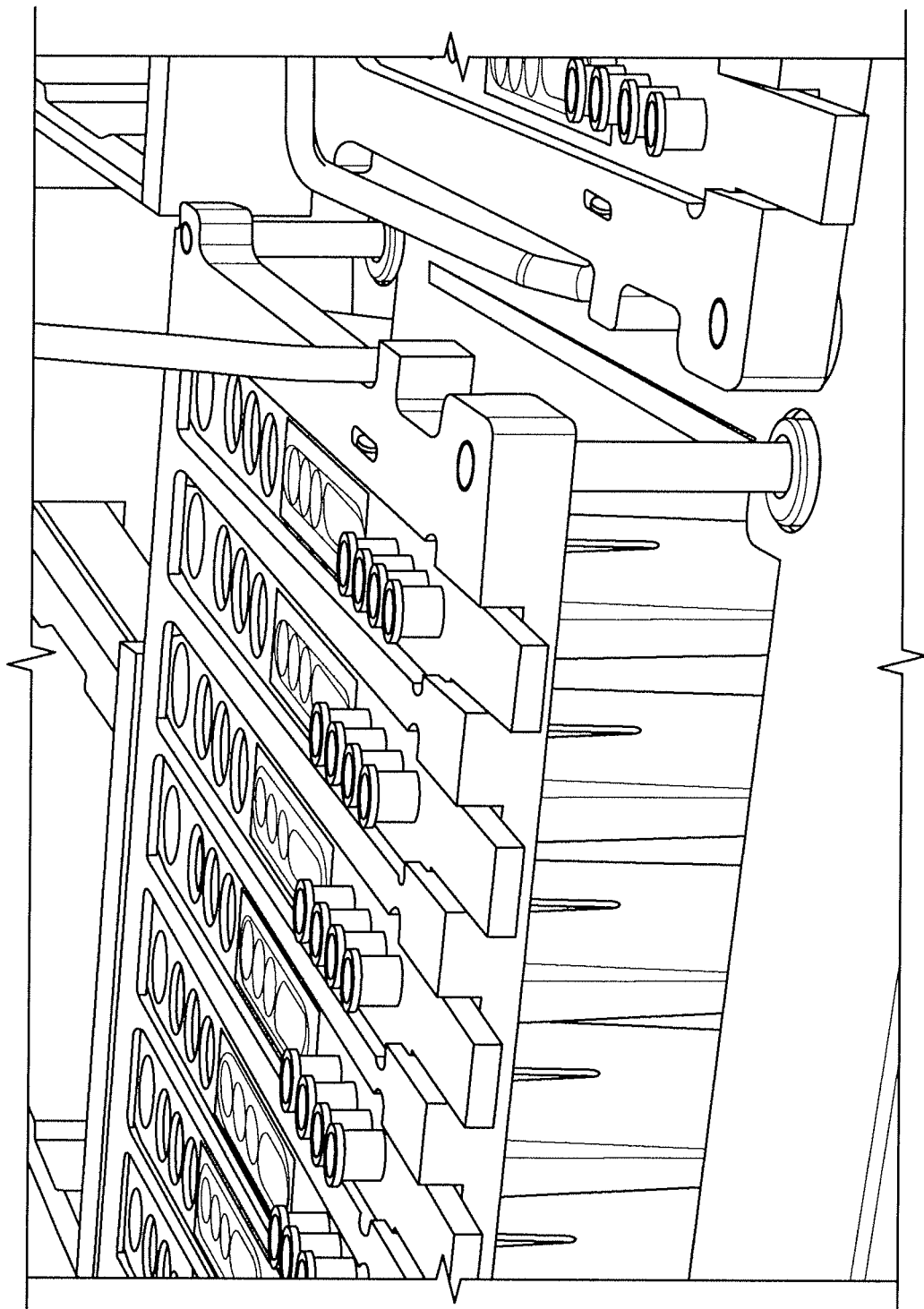


FIG. 8I

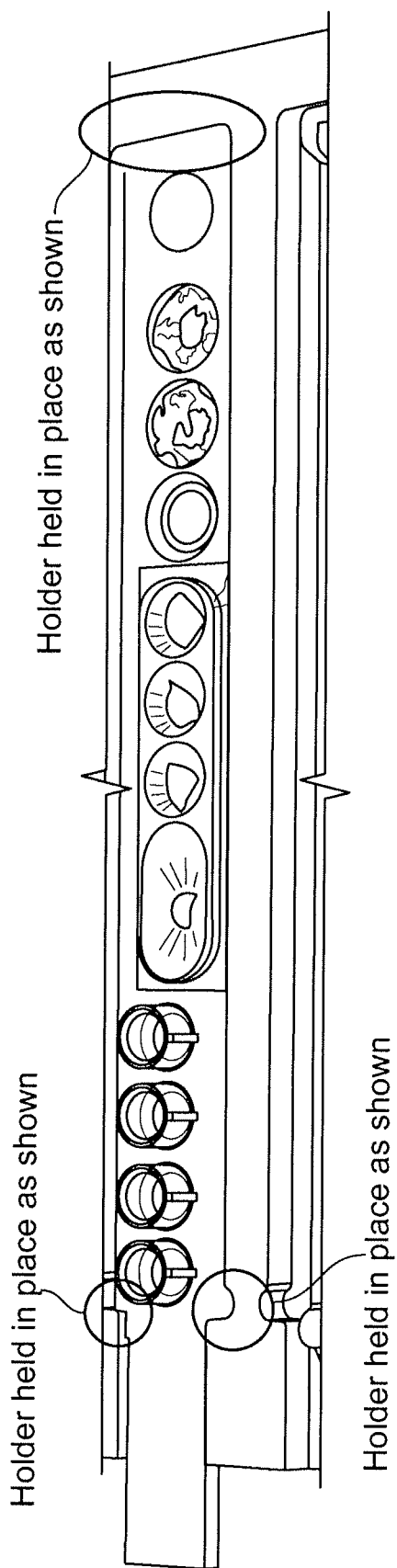


FIG. 8J

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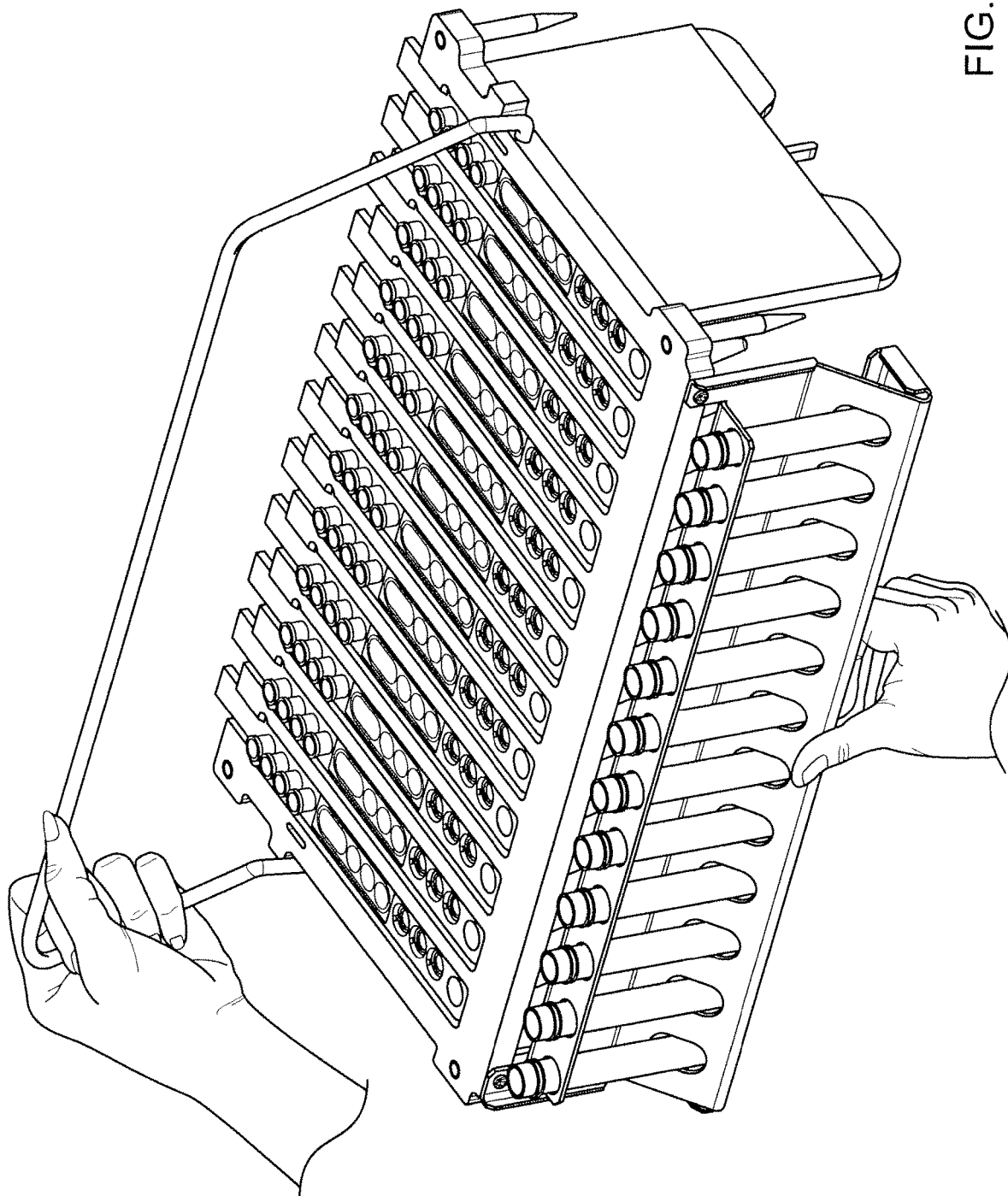


FIG. 8K

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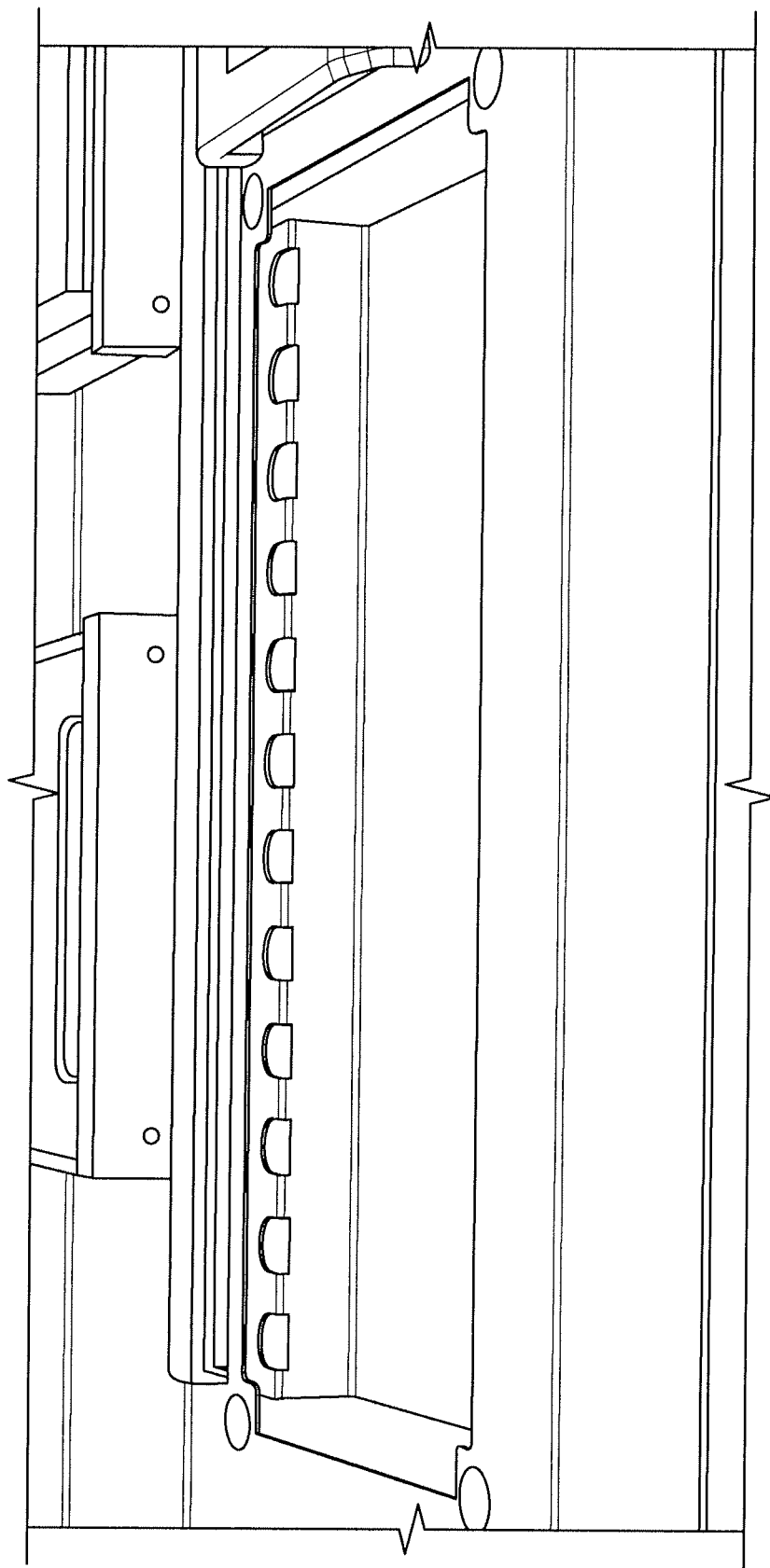


FIG. 9

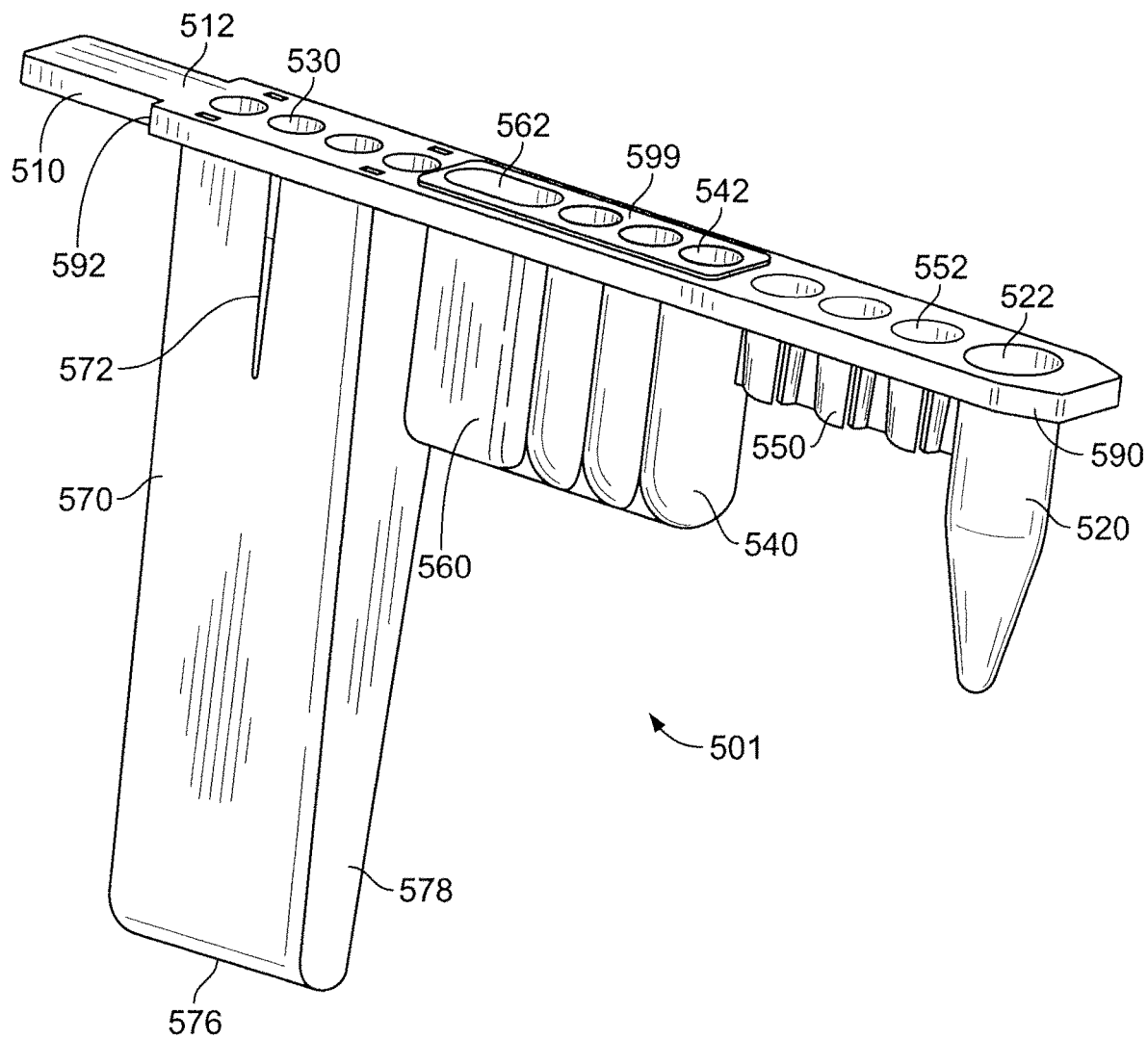


FIG. 10A

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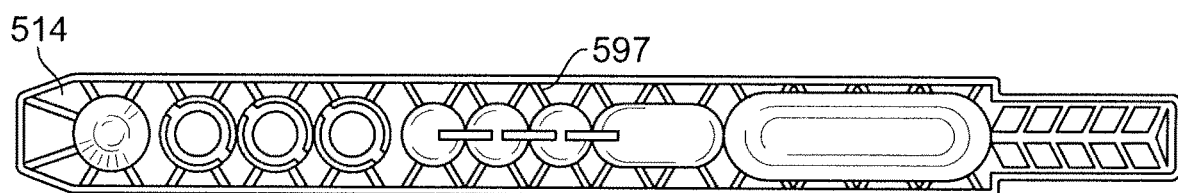


FIG. 10B

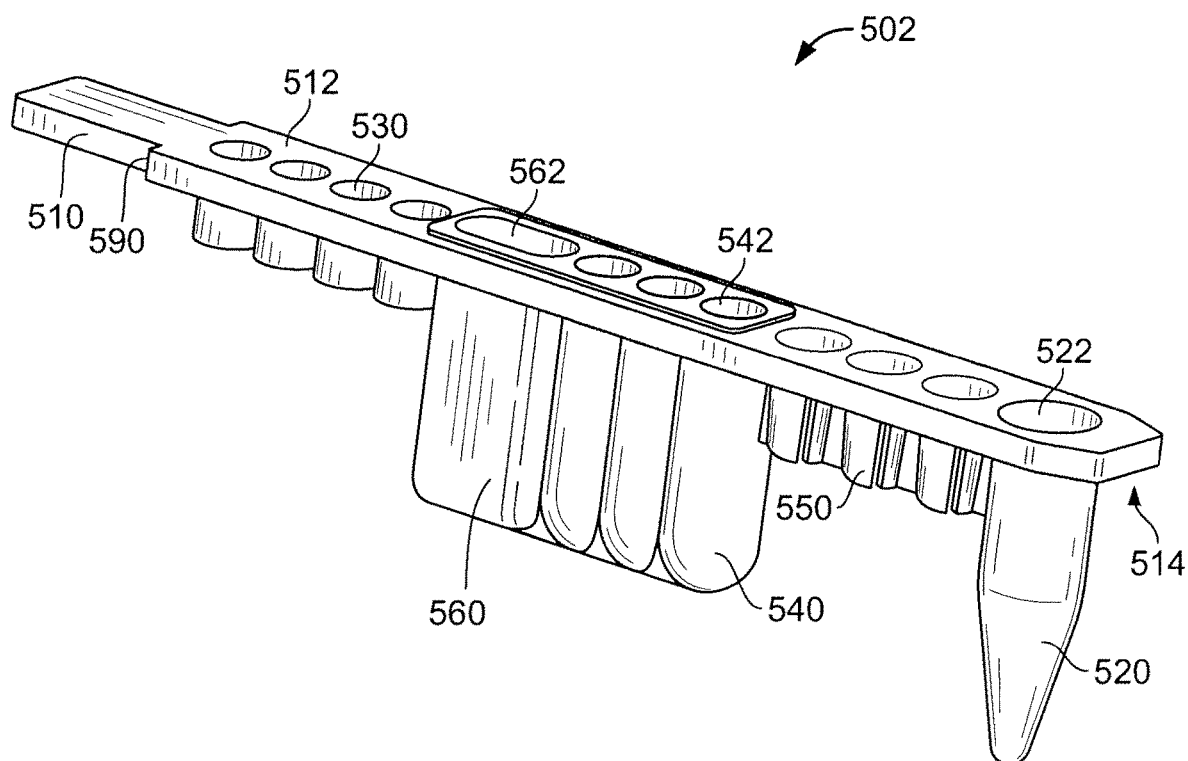


FIG. 11

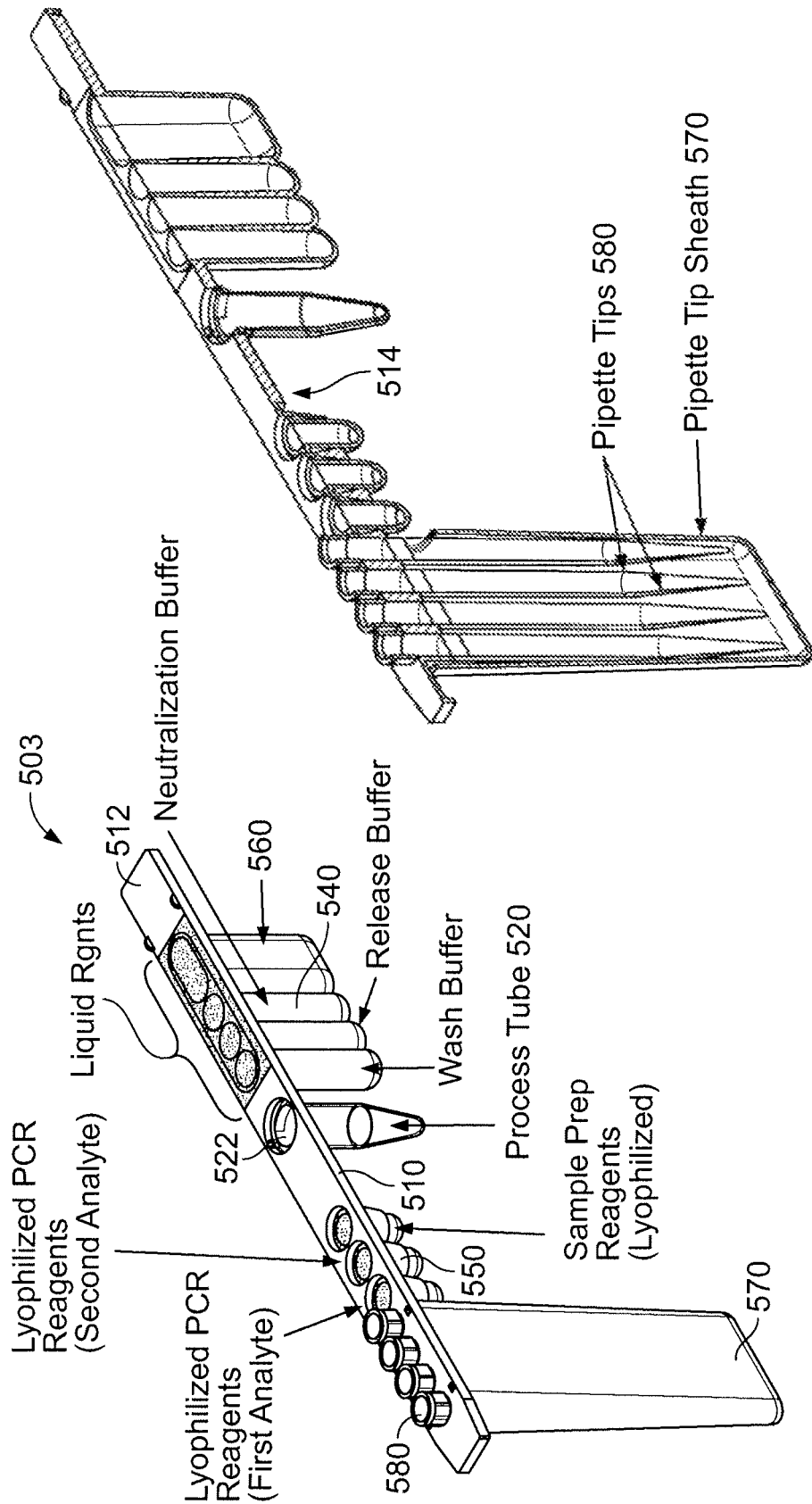


FIG. 12B

FIG. 12A

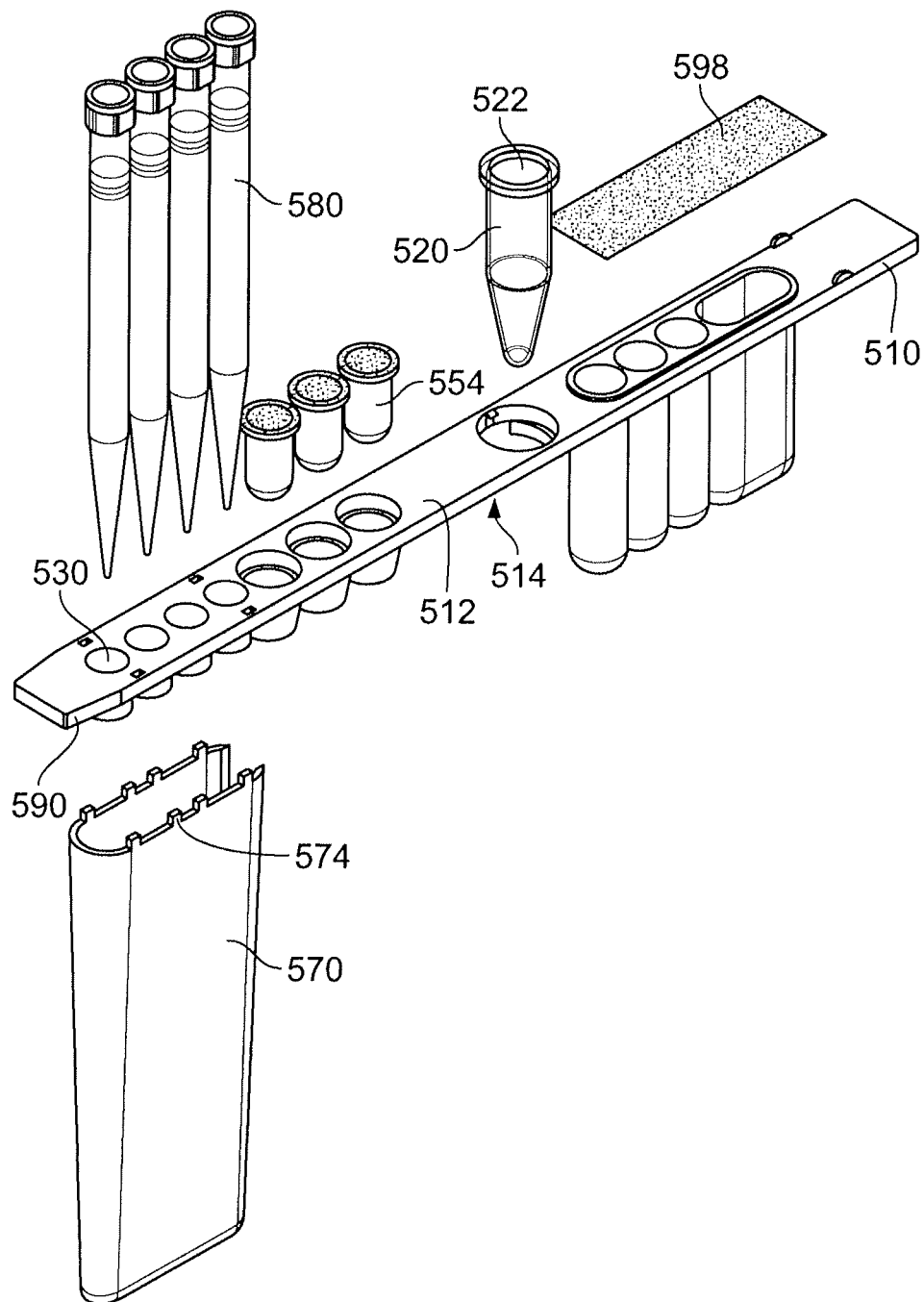


FIG. 12C

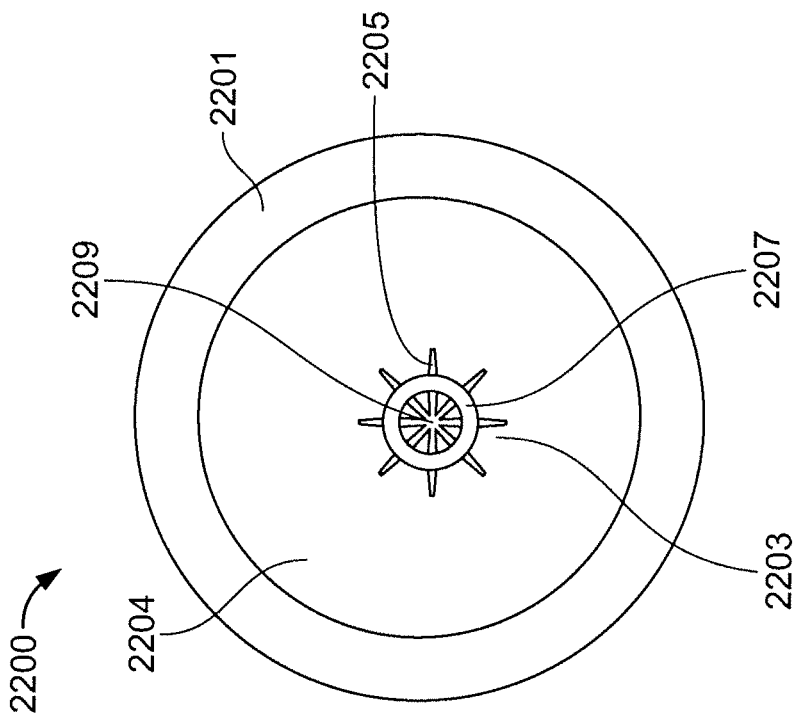


FIG. 13B

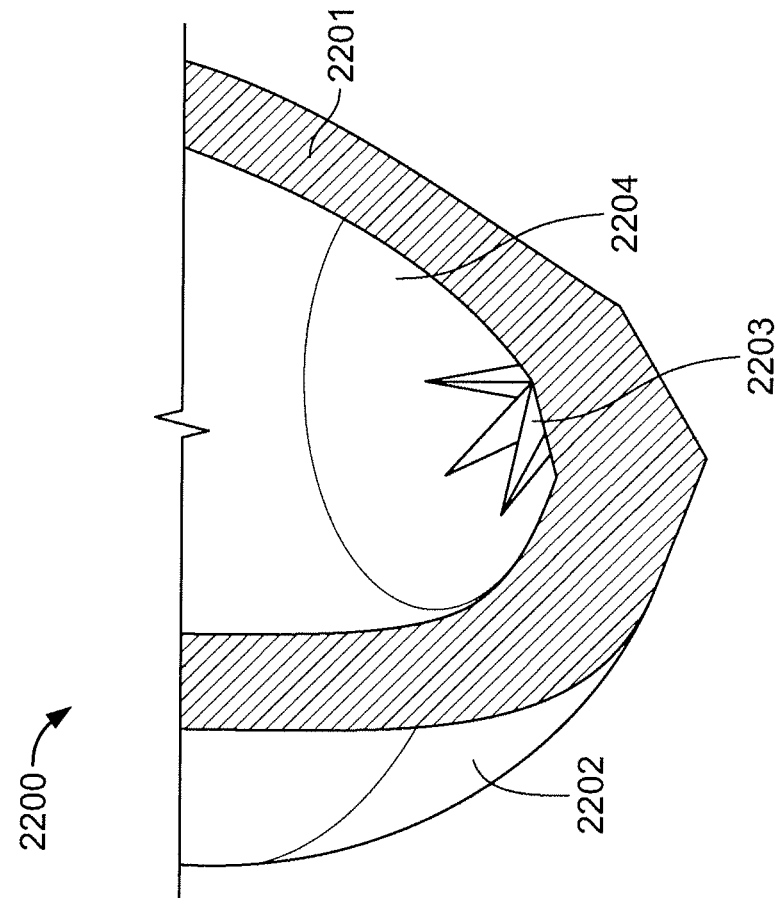


FIG. 13A

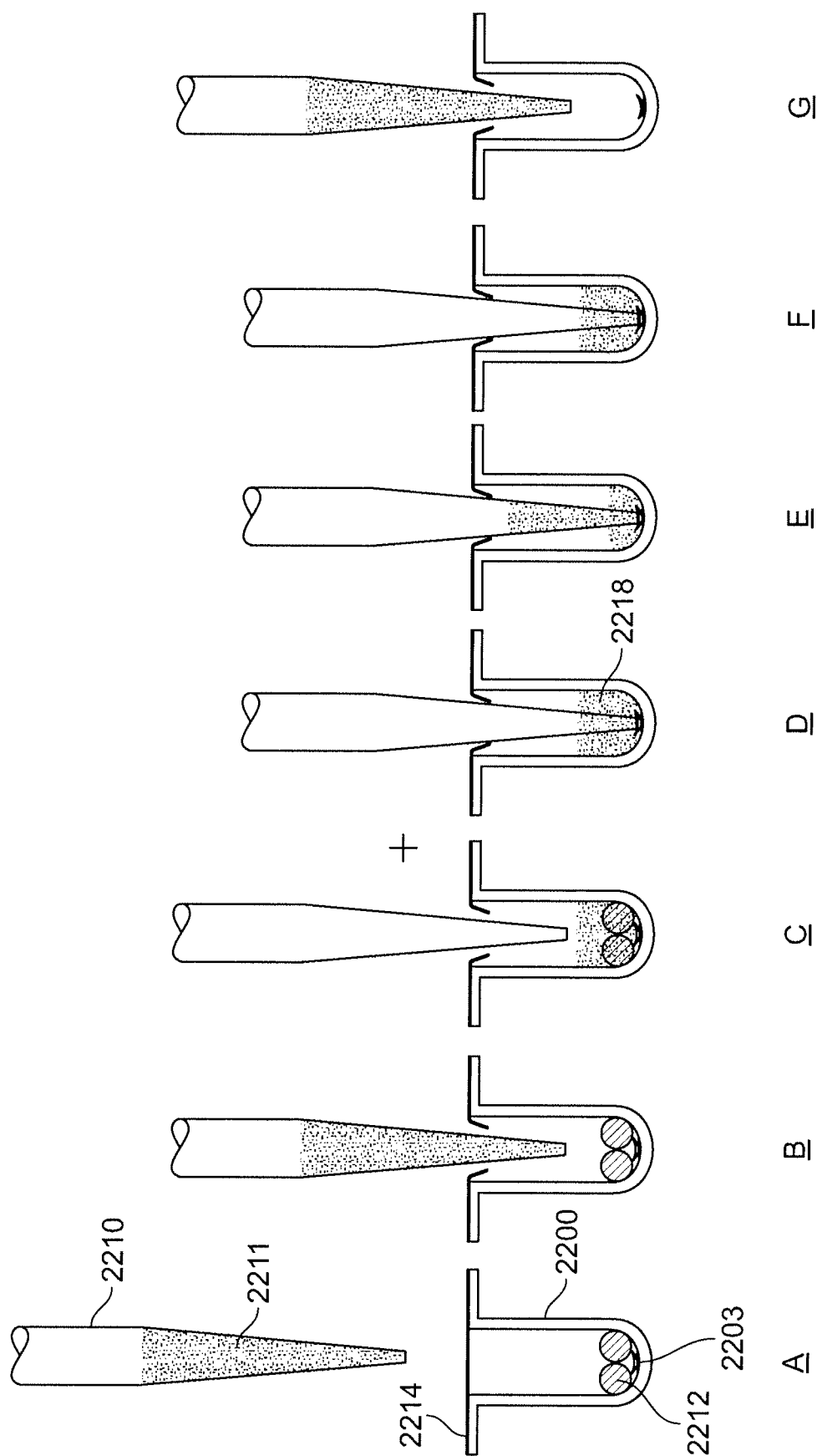
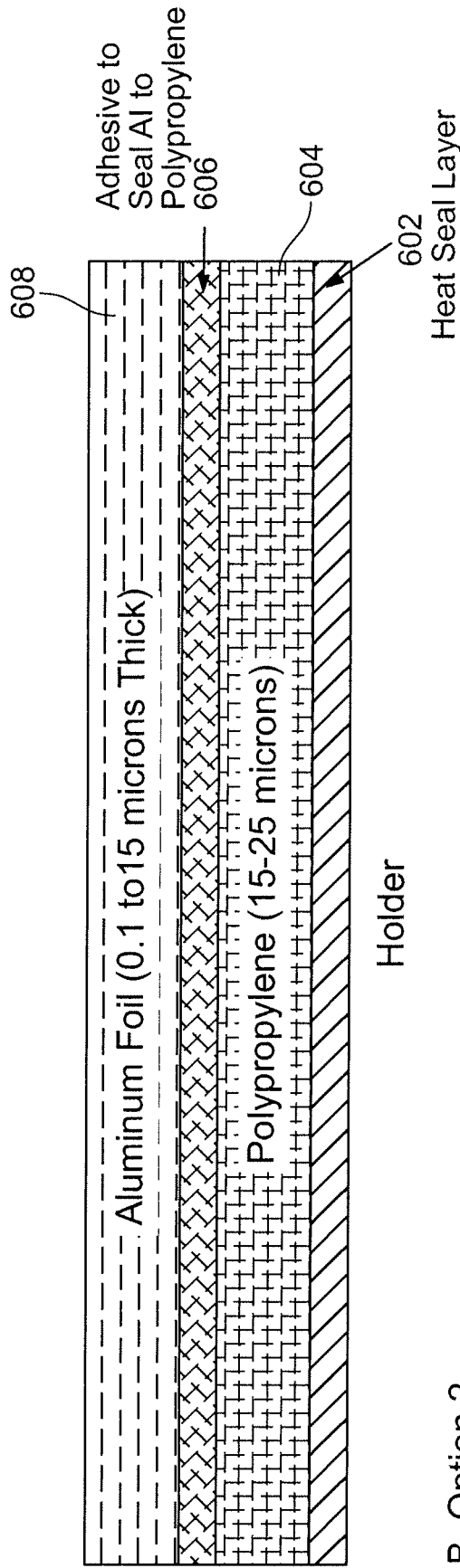


FIG. 14

A. Option 1



B. Option 2

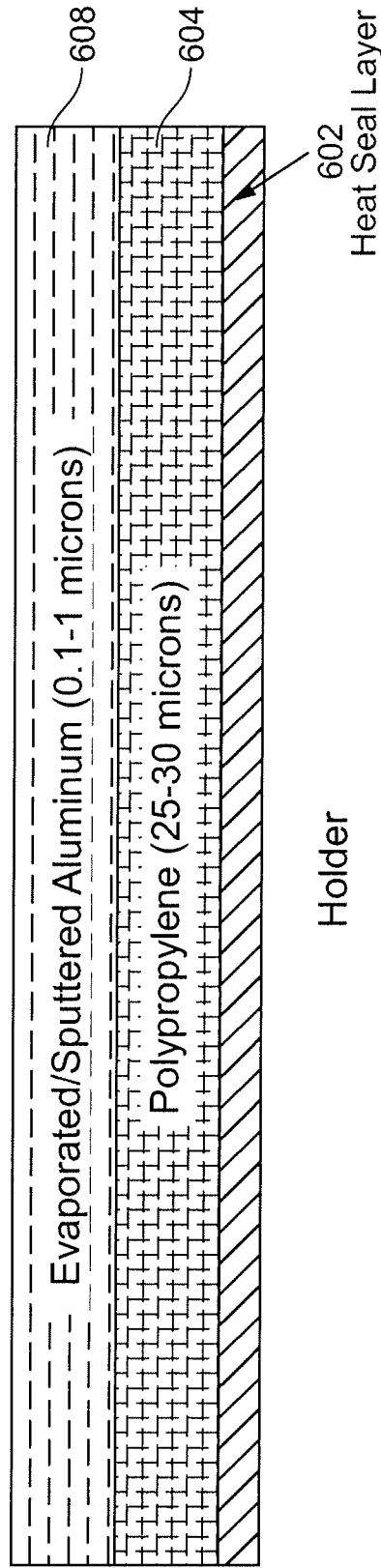


FIG. 15

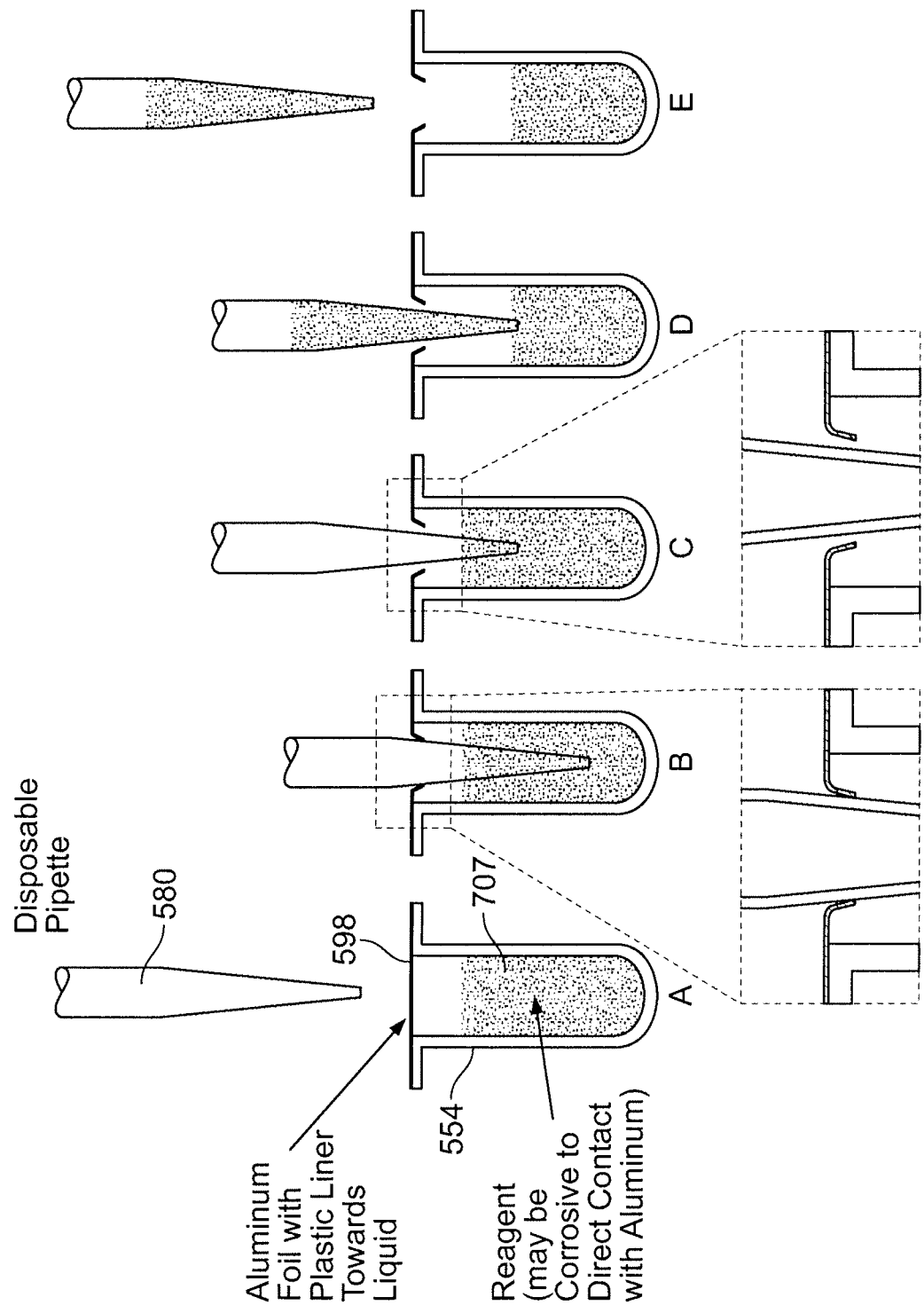


FIG. 16

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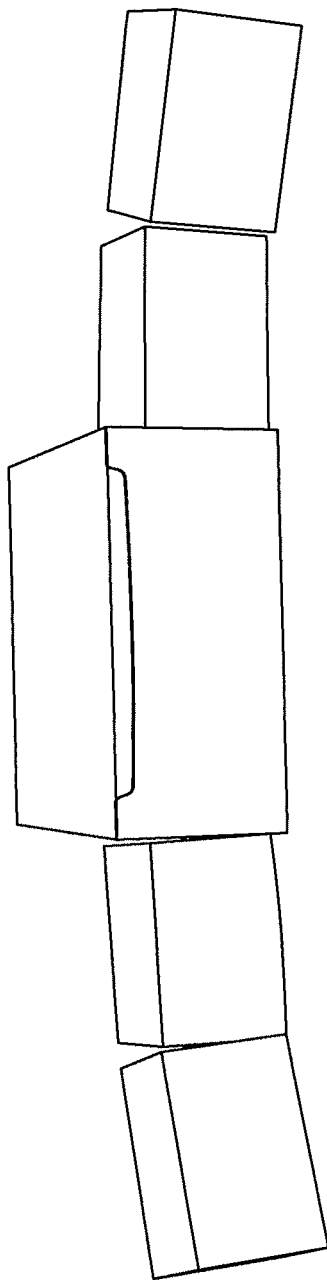


FIG. 17A

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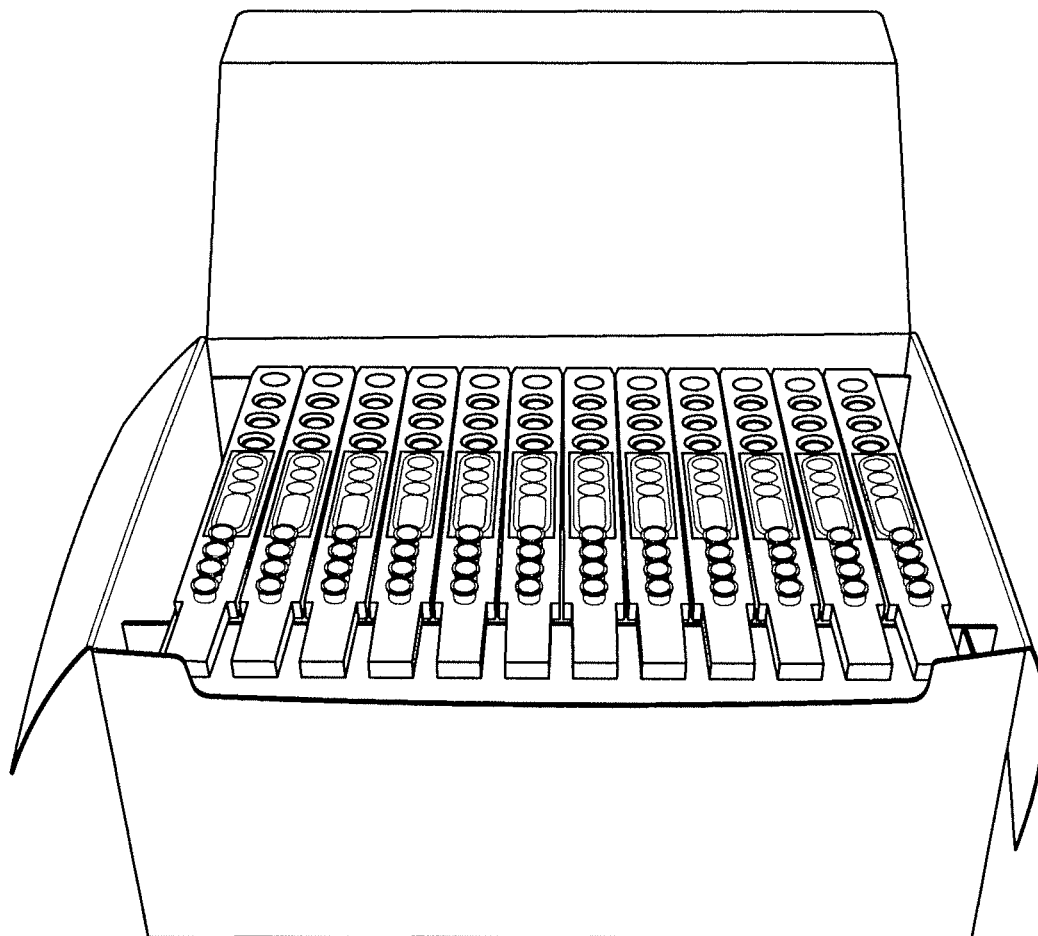


FIG. 17B

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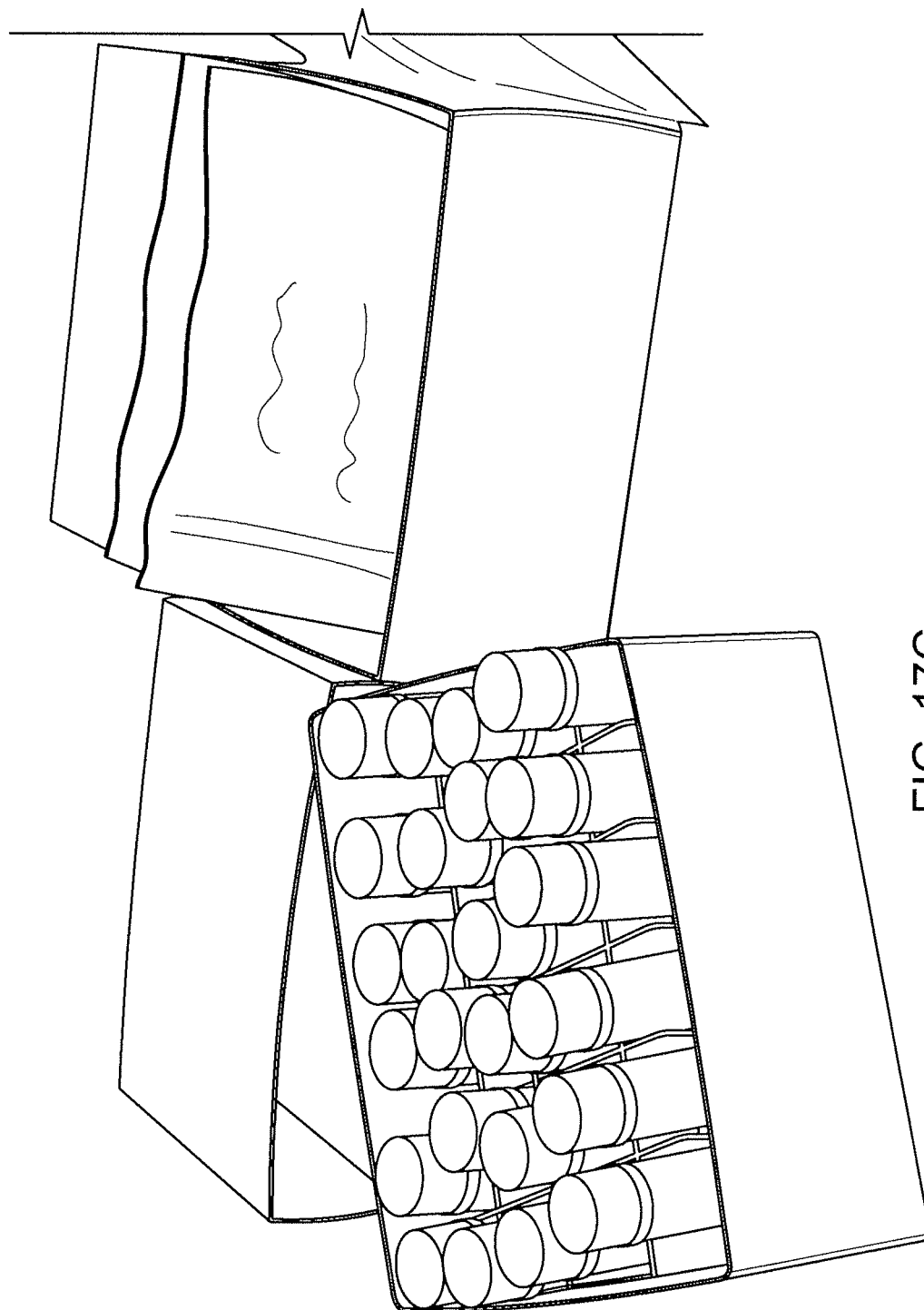


FIG. 17C

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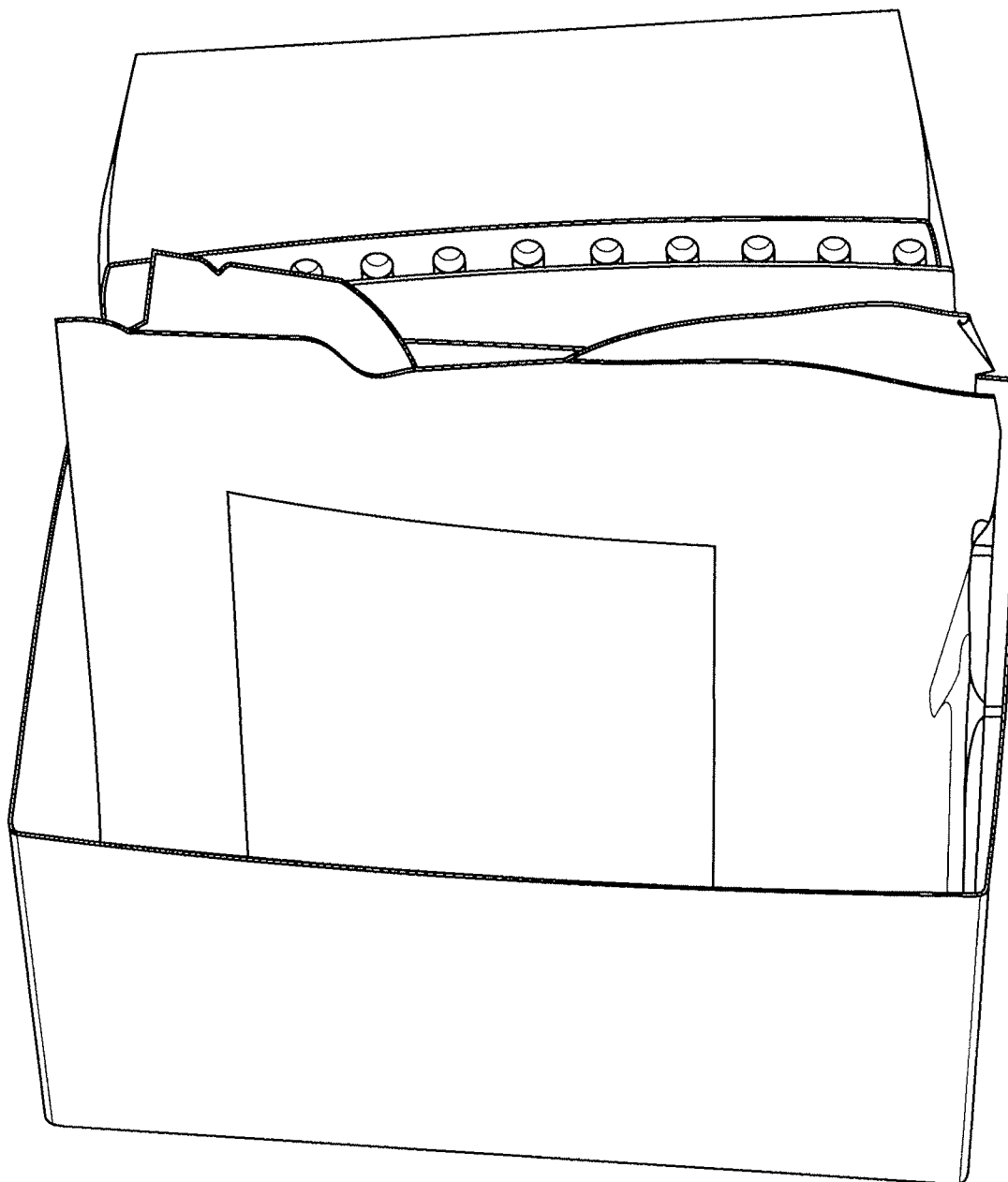
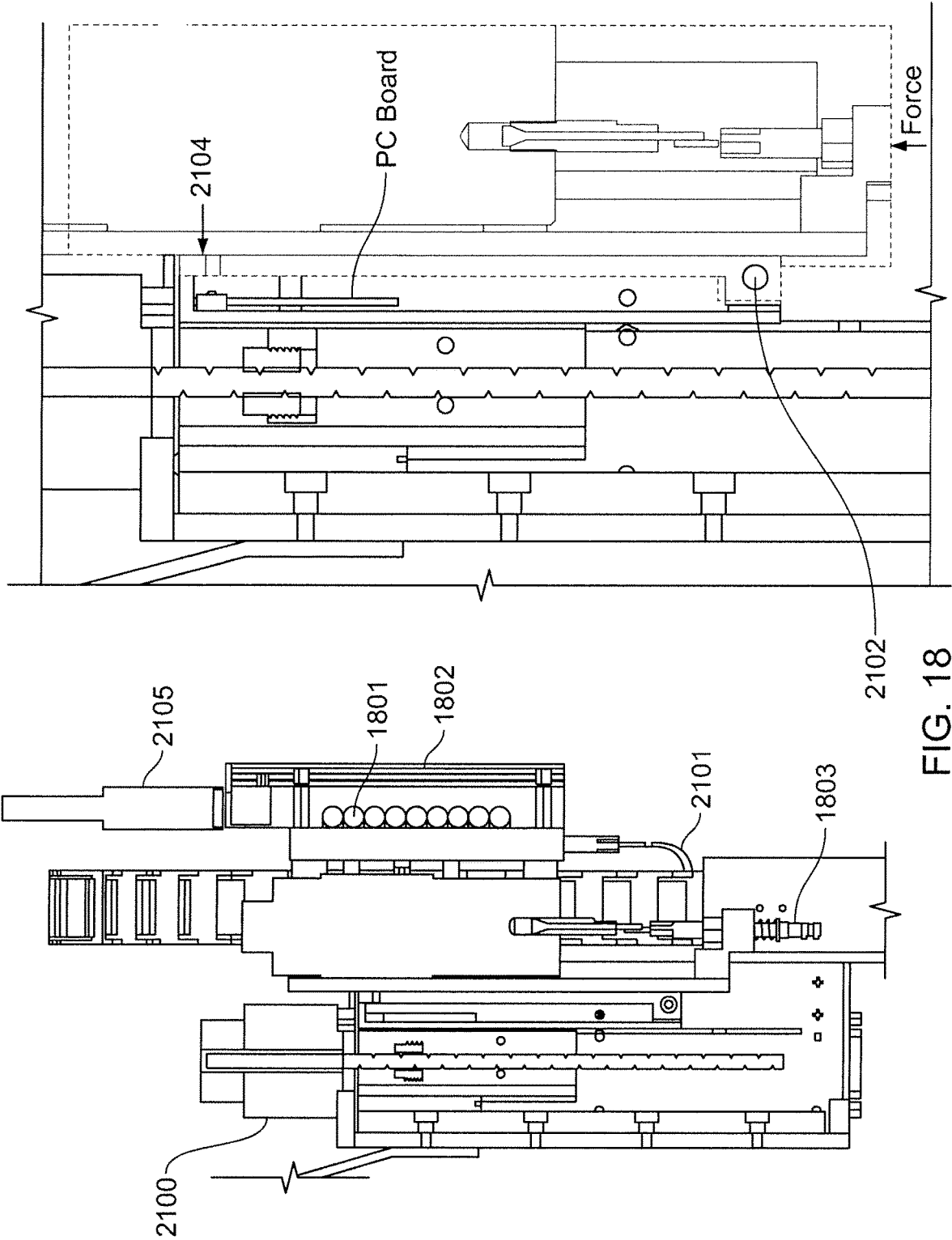


FIG. 17D



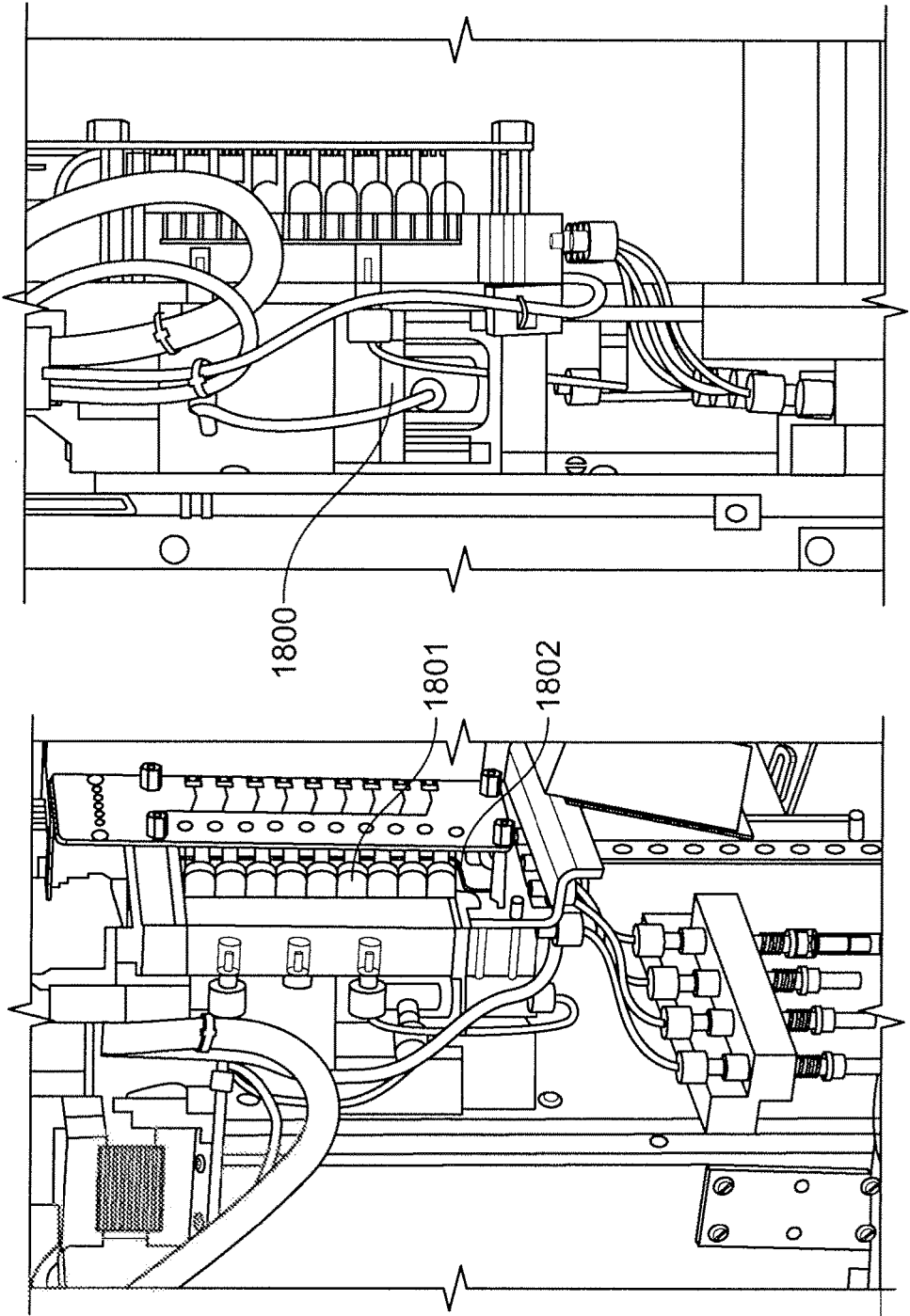
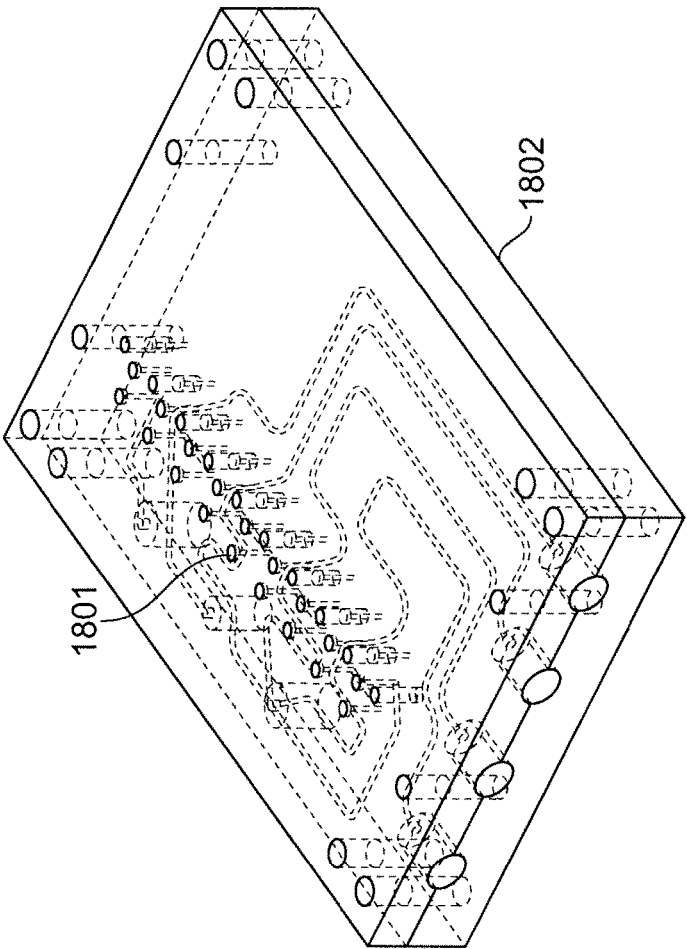
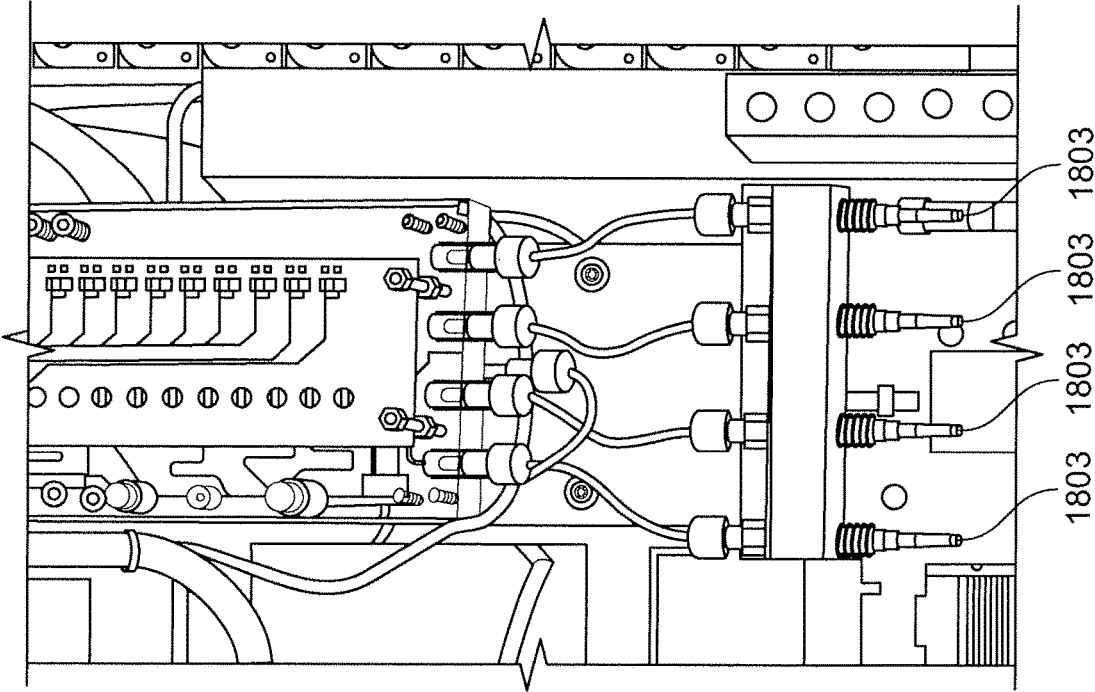


FIG. 19B

FIG. 19A



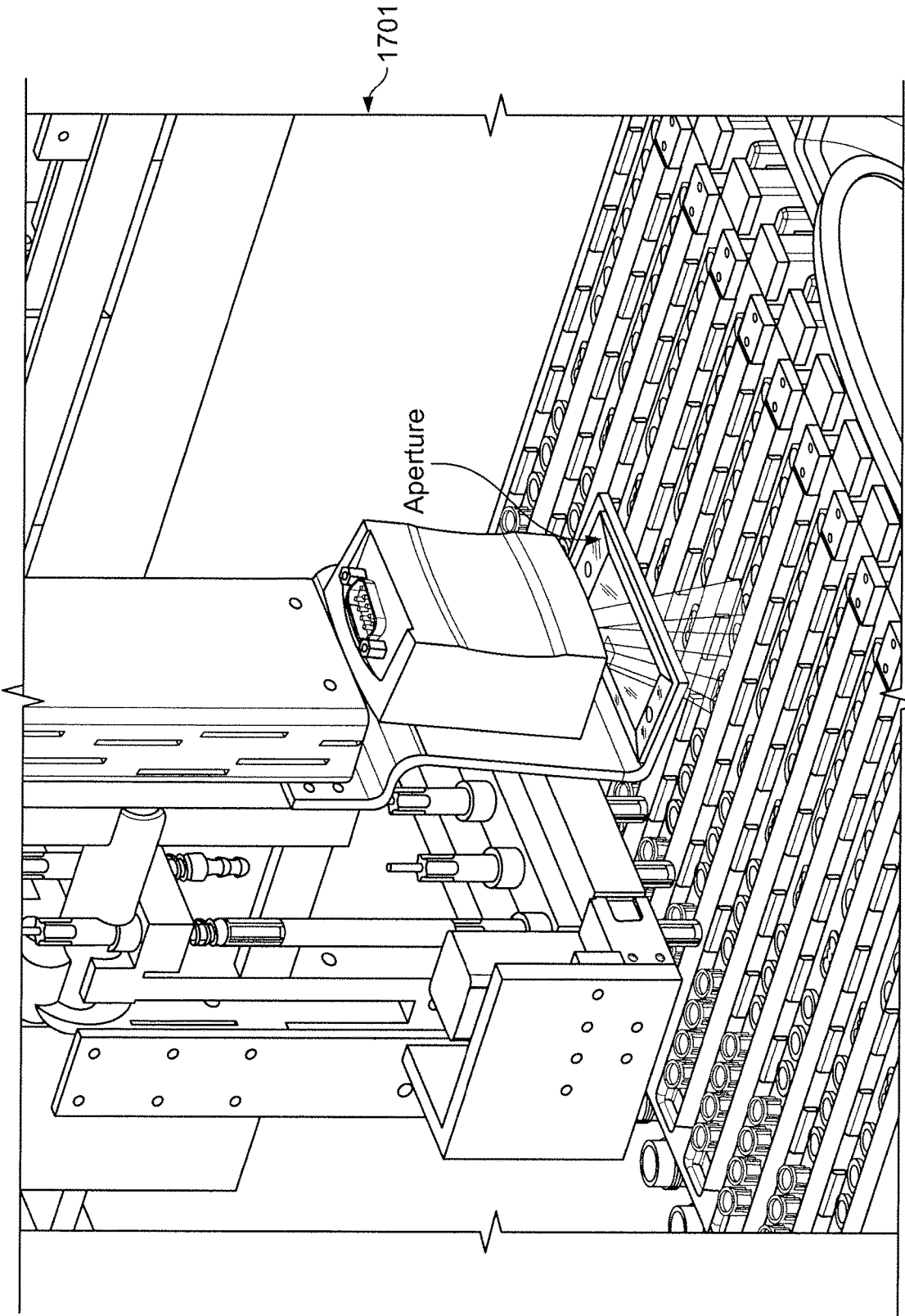


FIG. 21

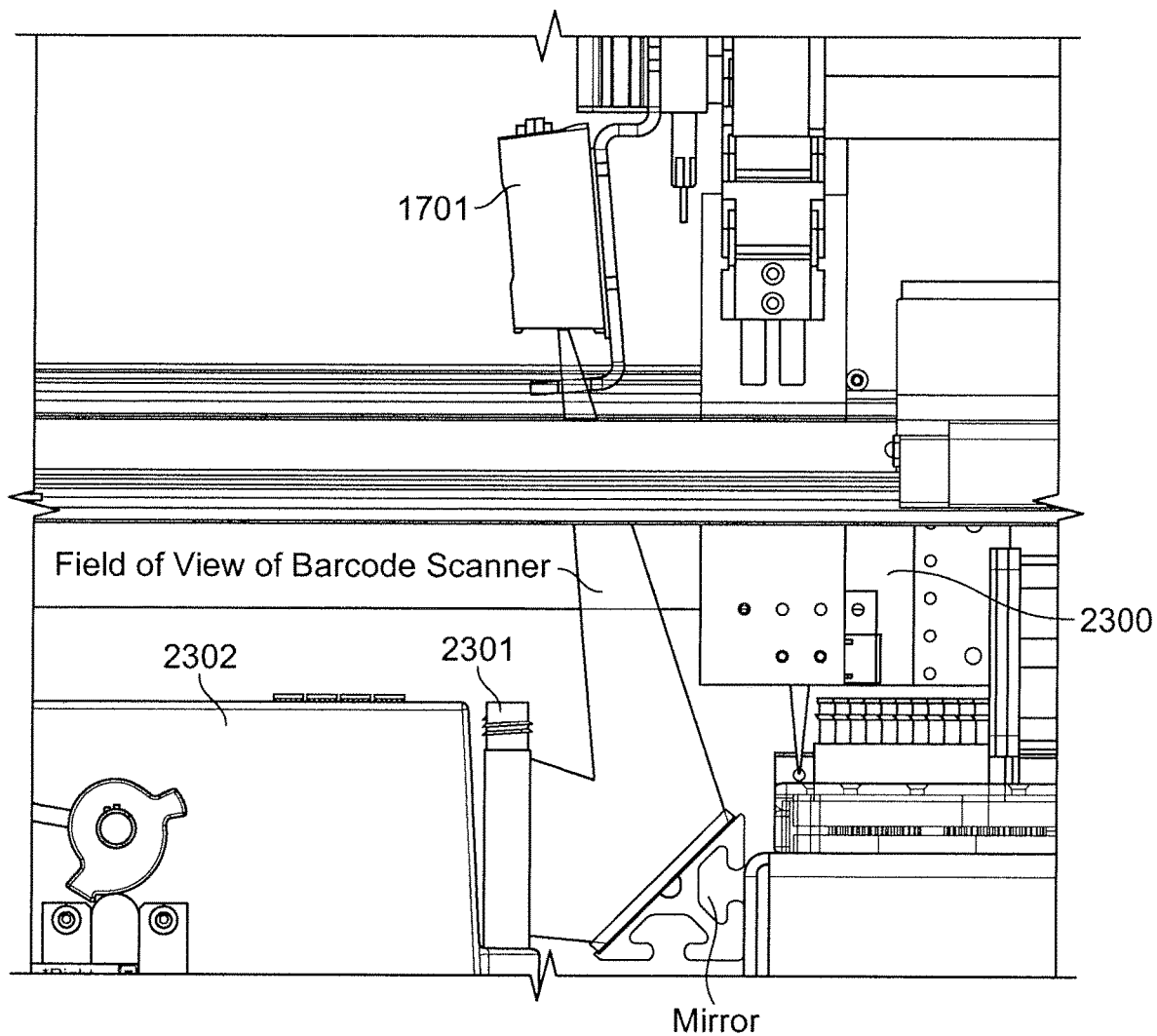


FIG. 22

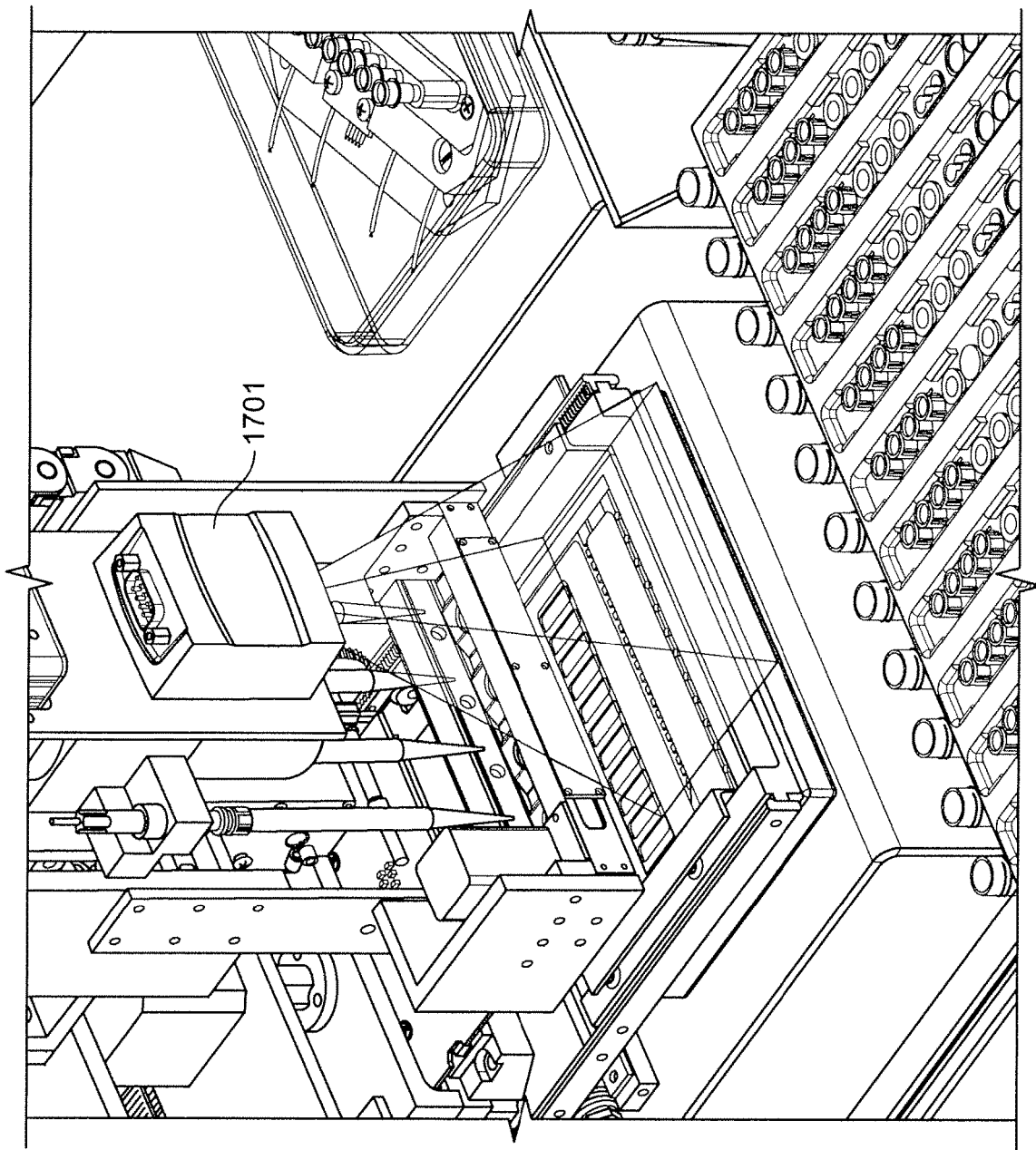
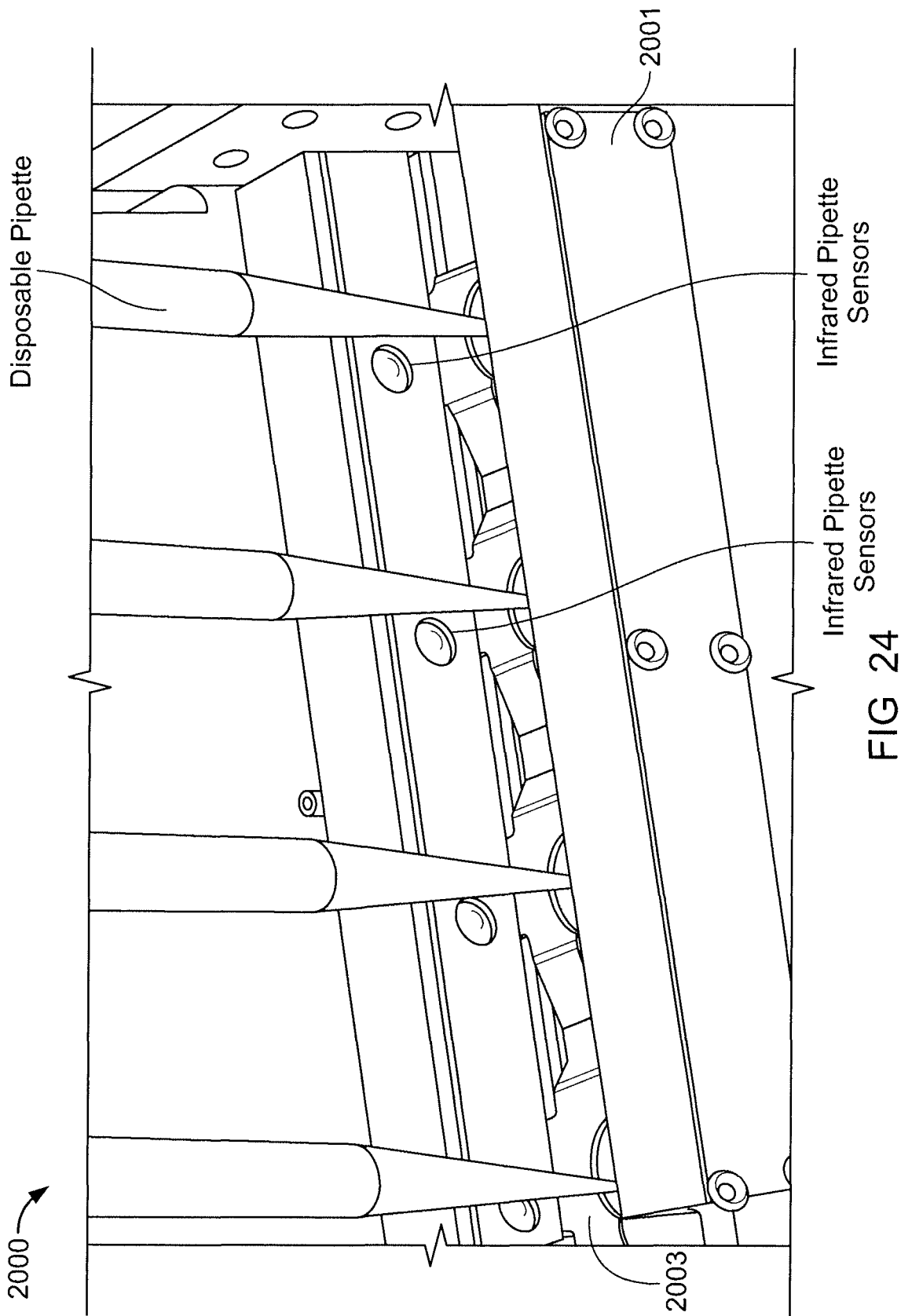
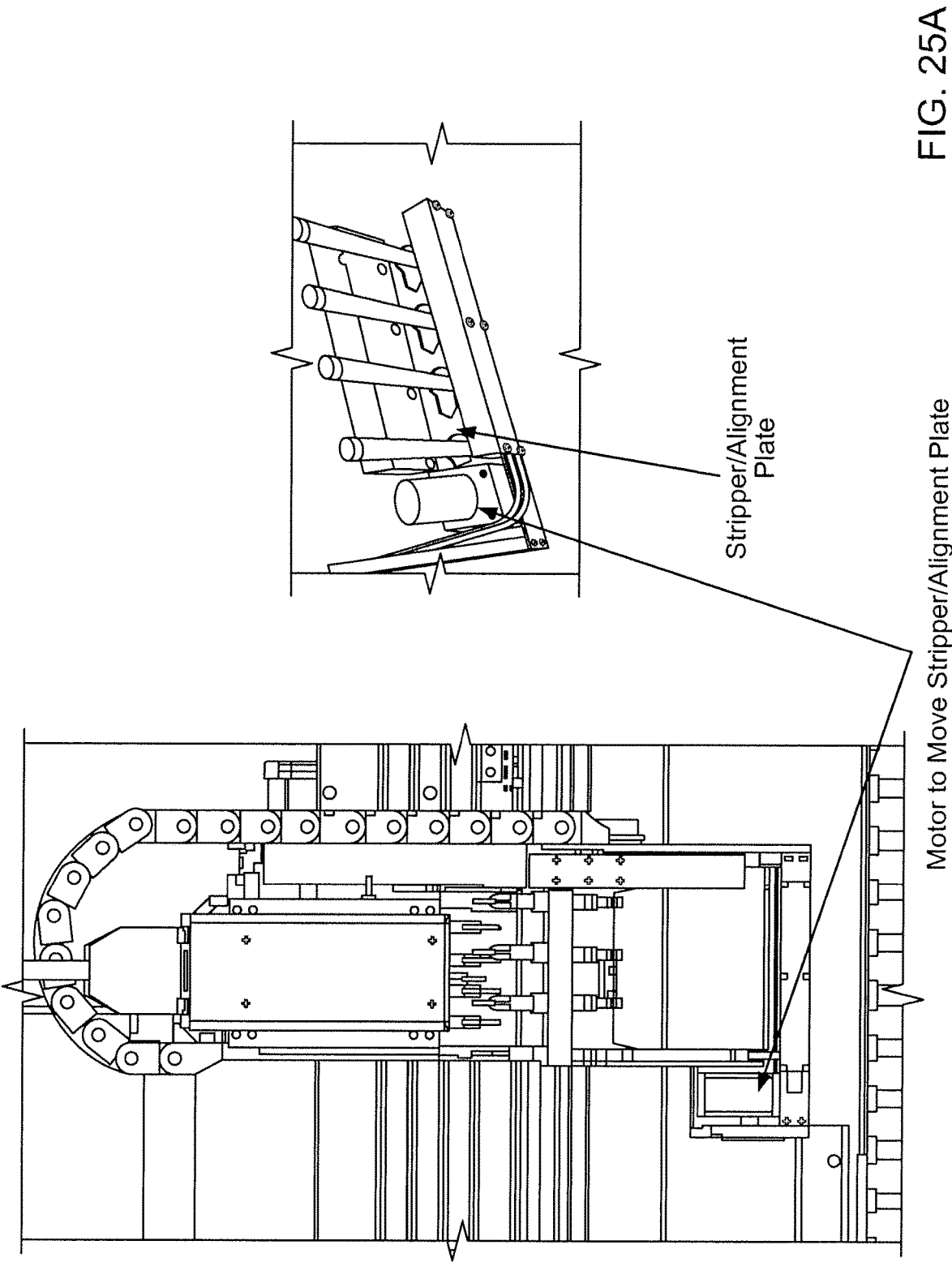


FIG. 23





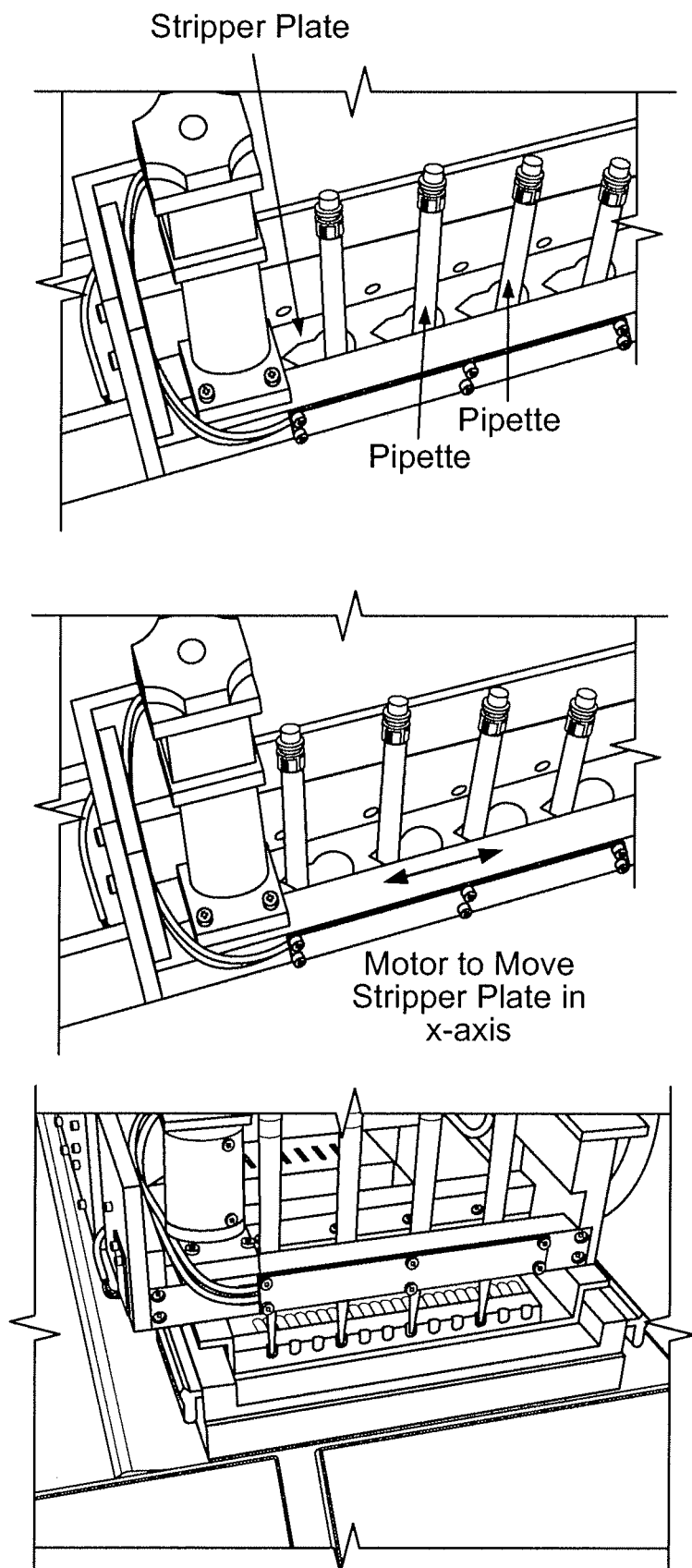
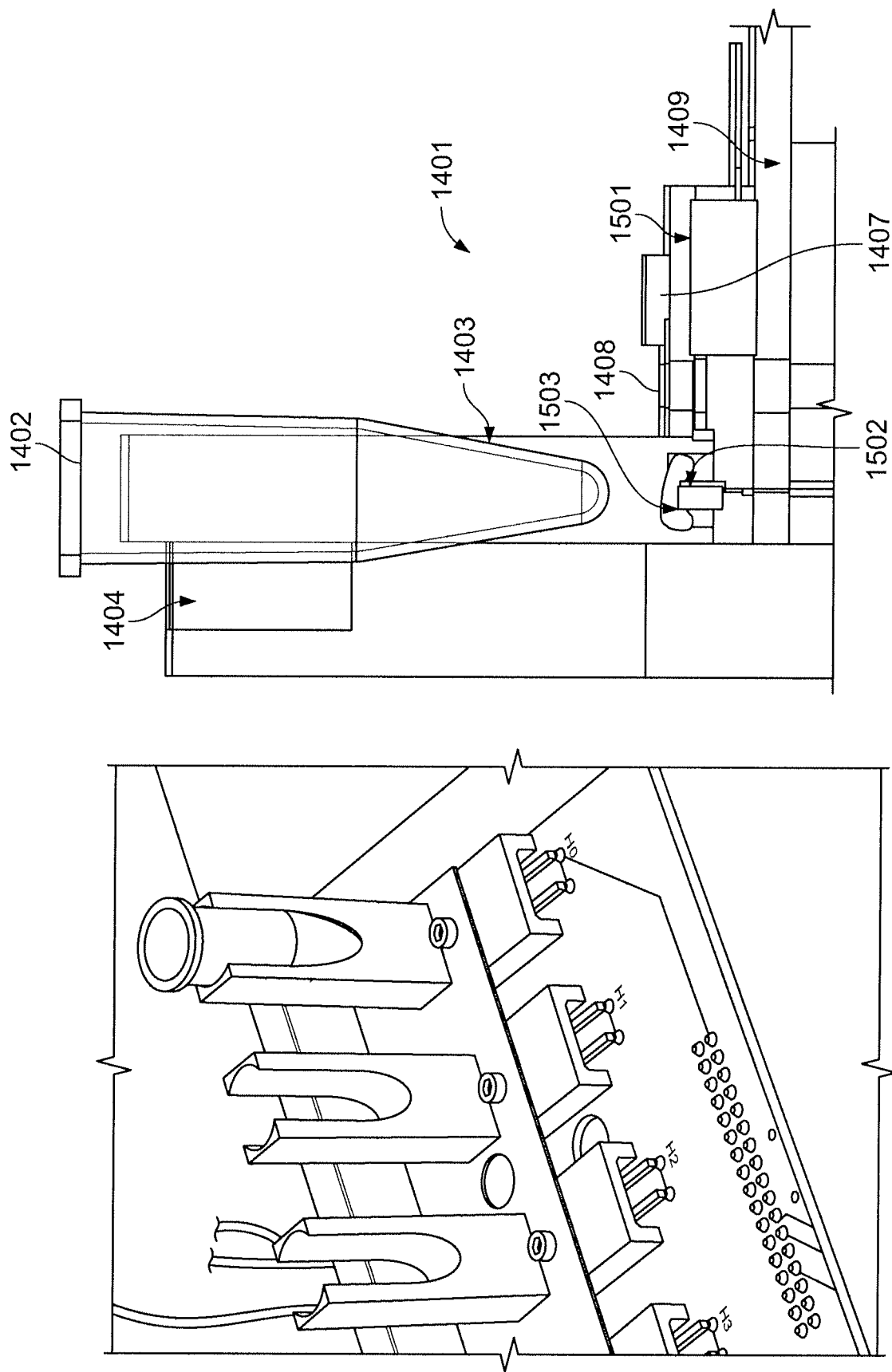


FIG. 25B



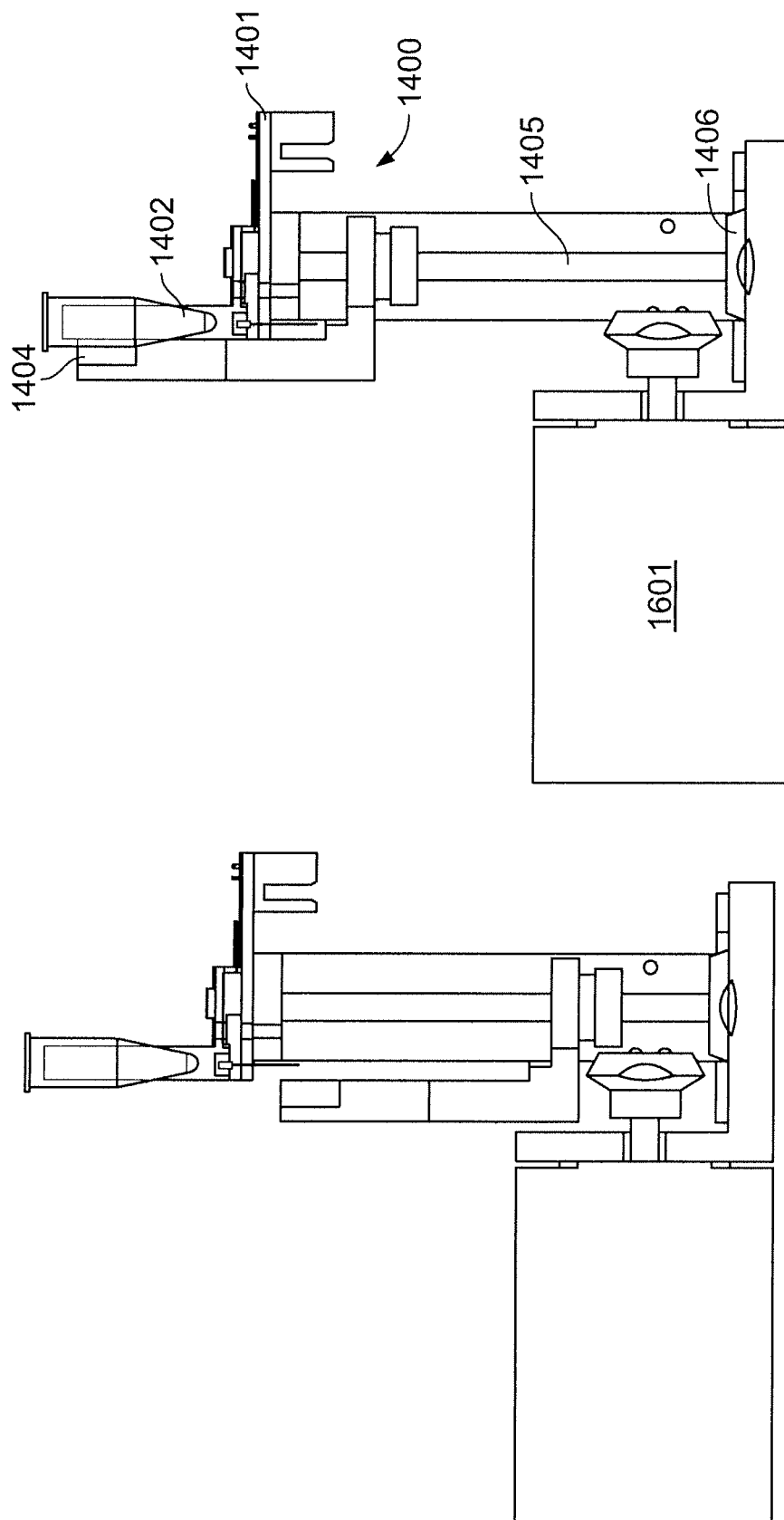


FIG. 27

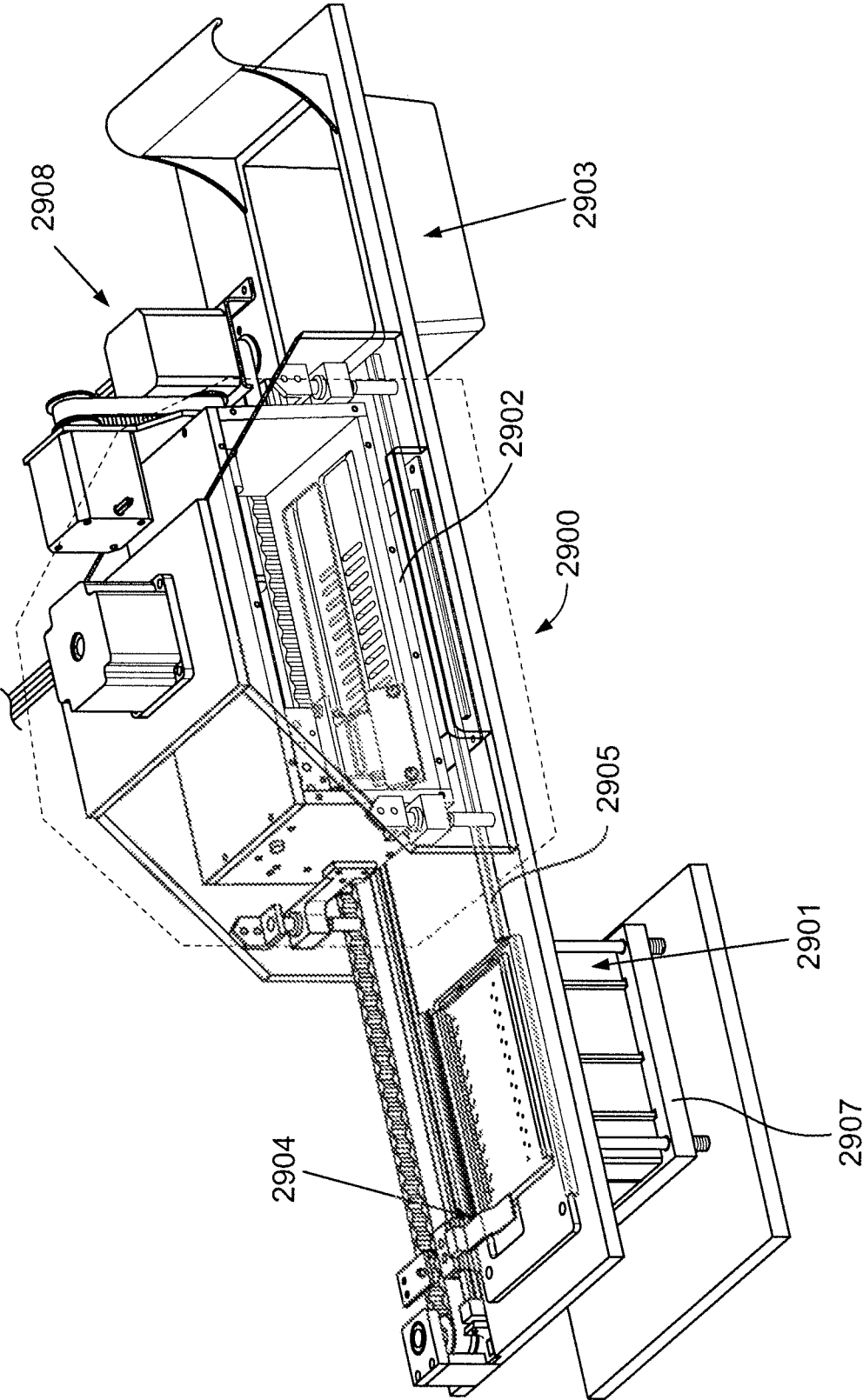


FIG. 28

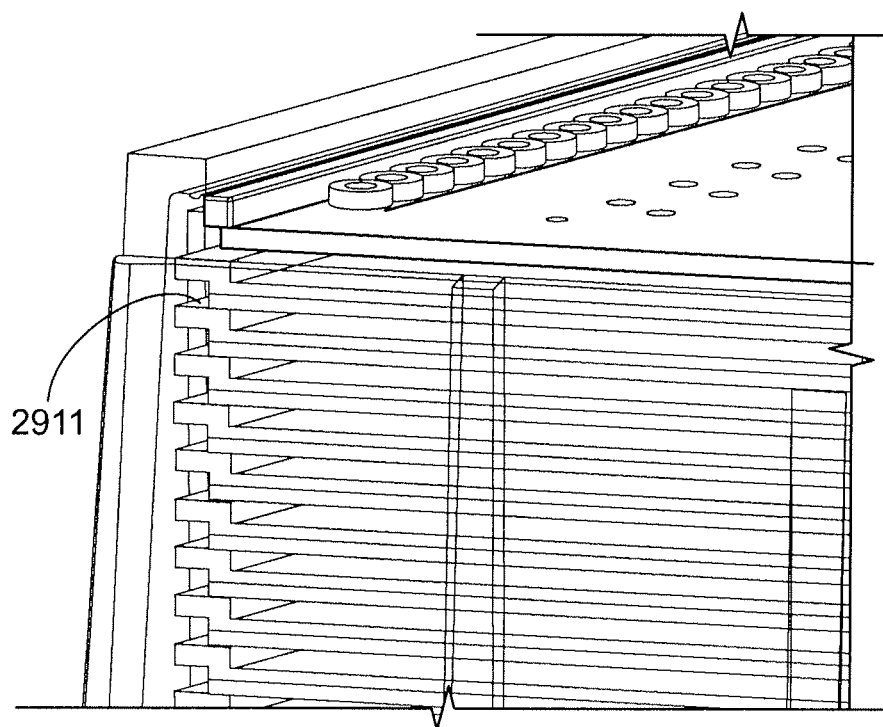
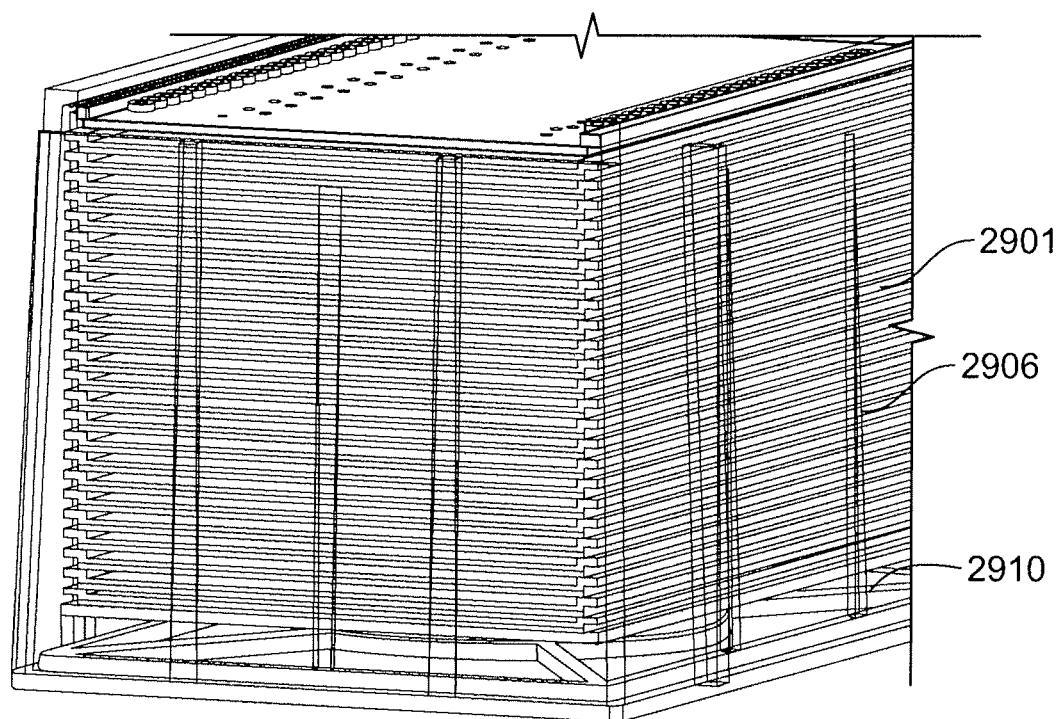


FIG. 29

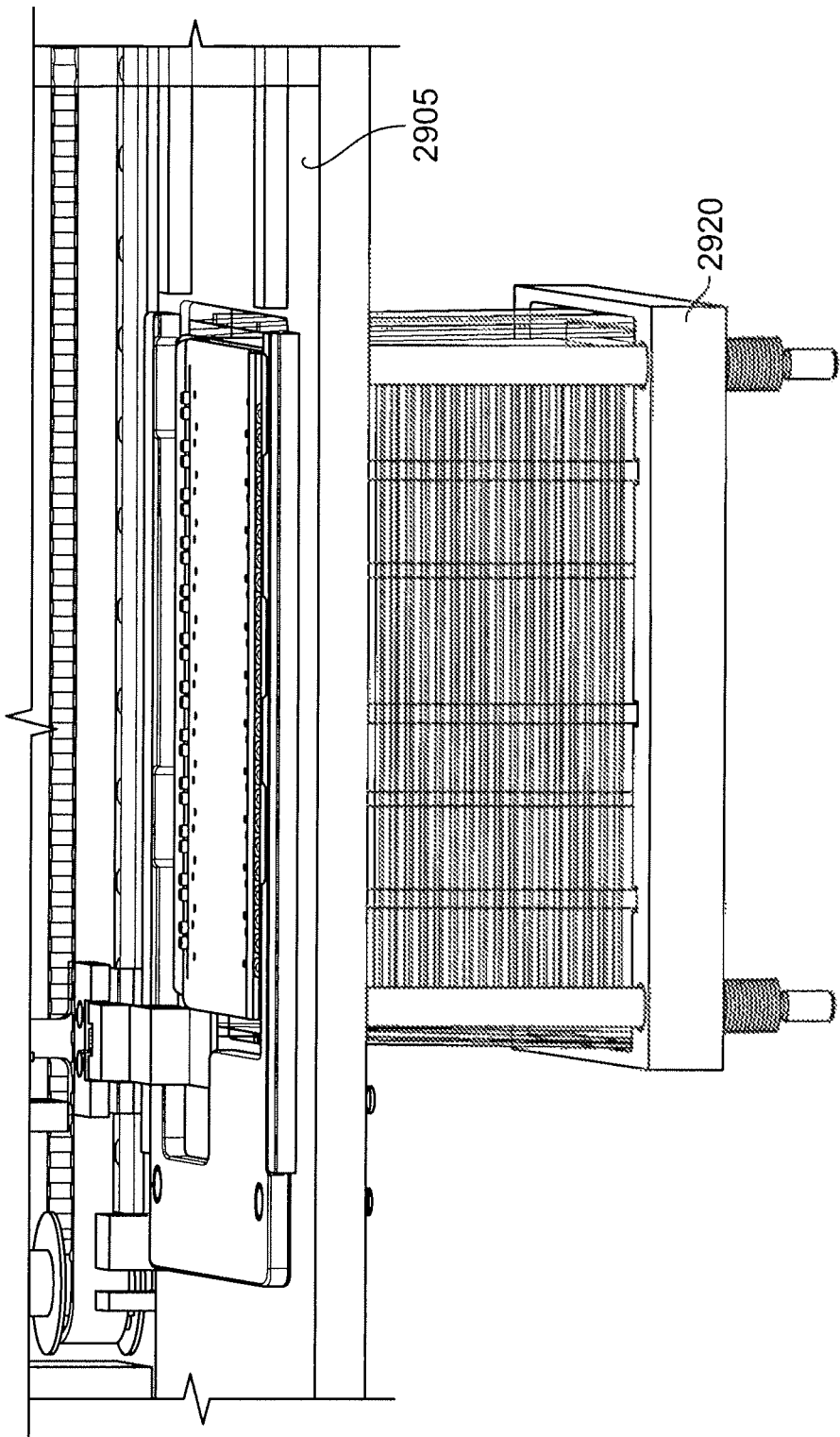


FIG. 30

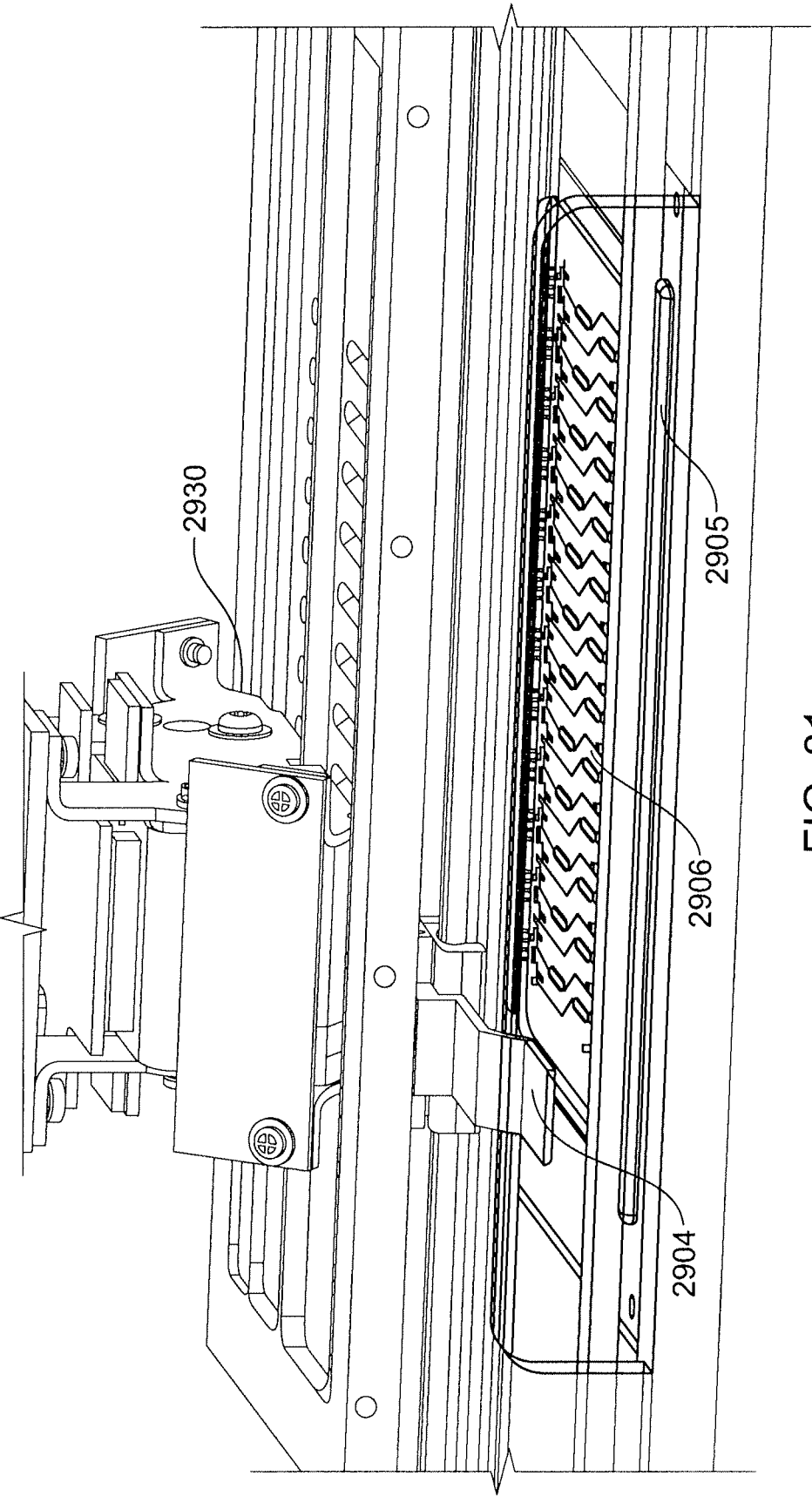


FIG. 31

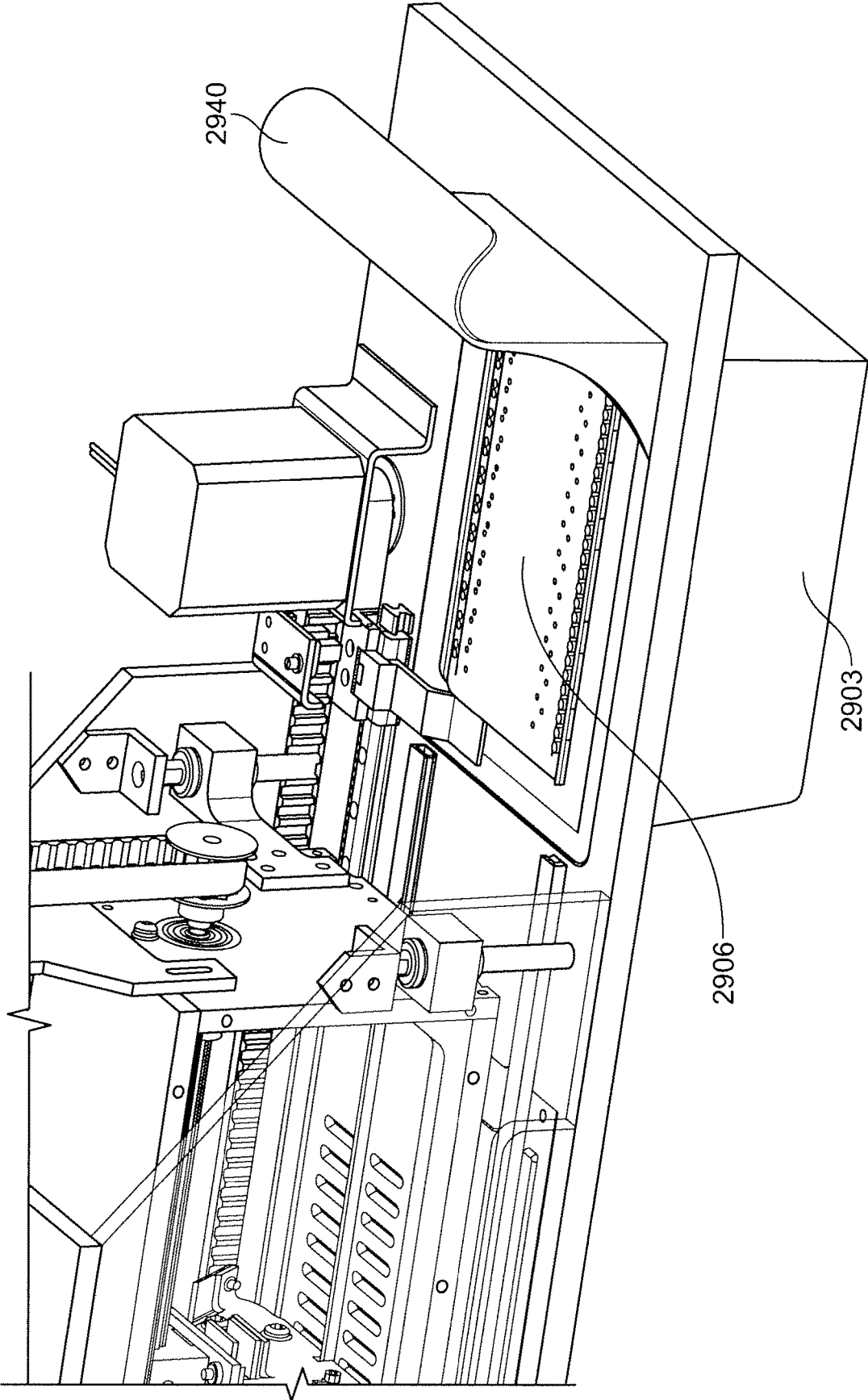


FIG. 32

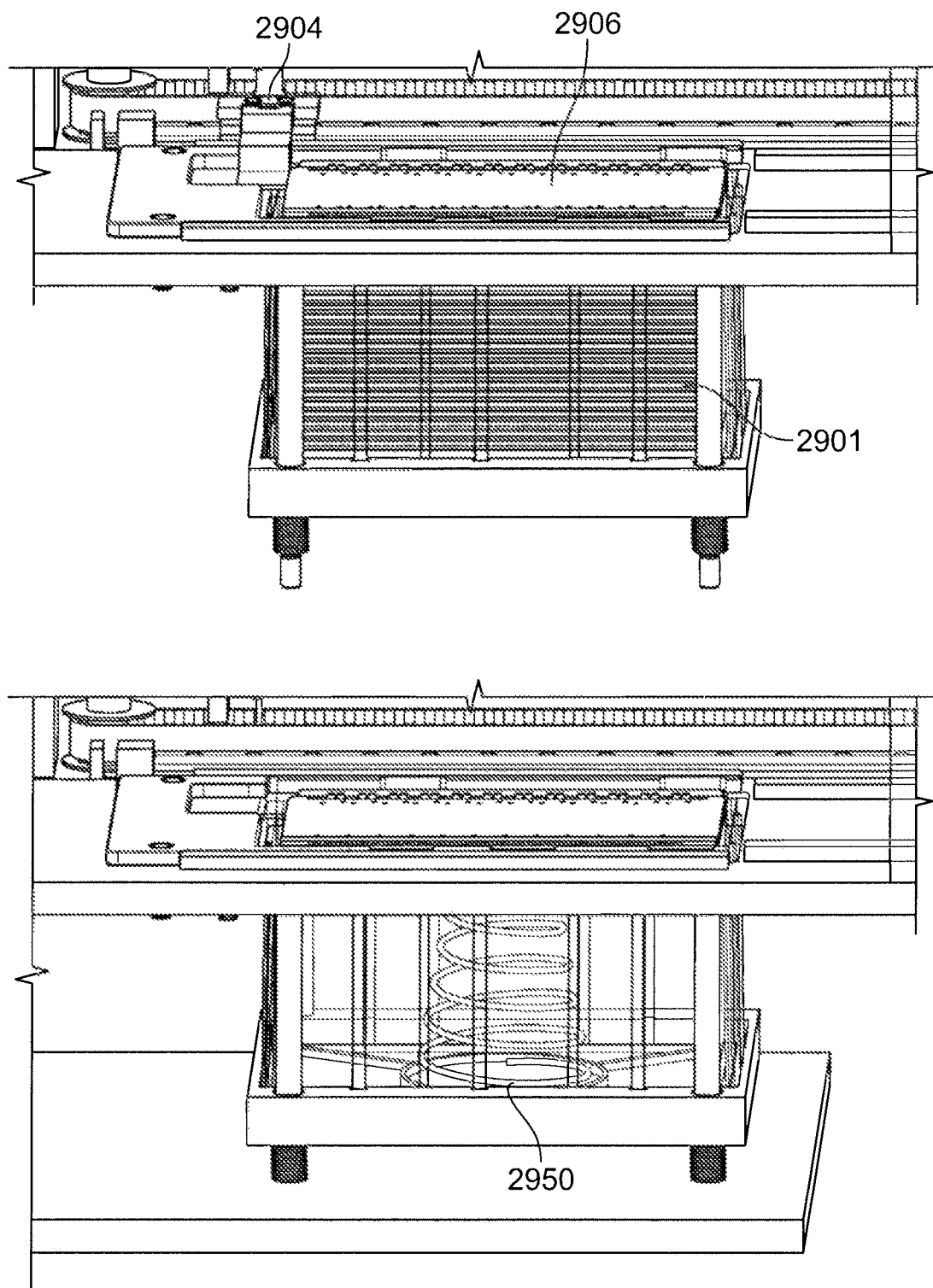
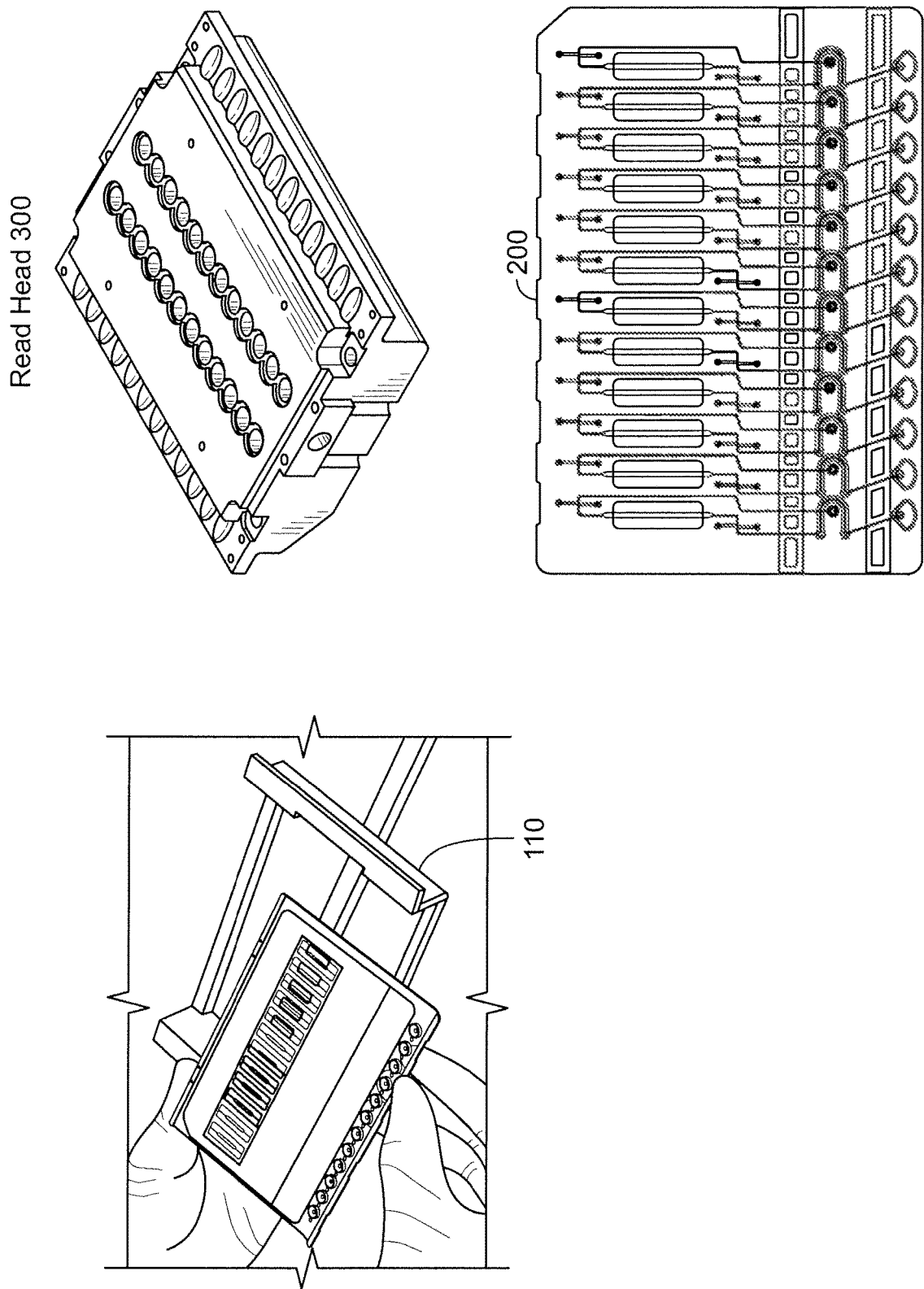


FIG. 33



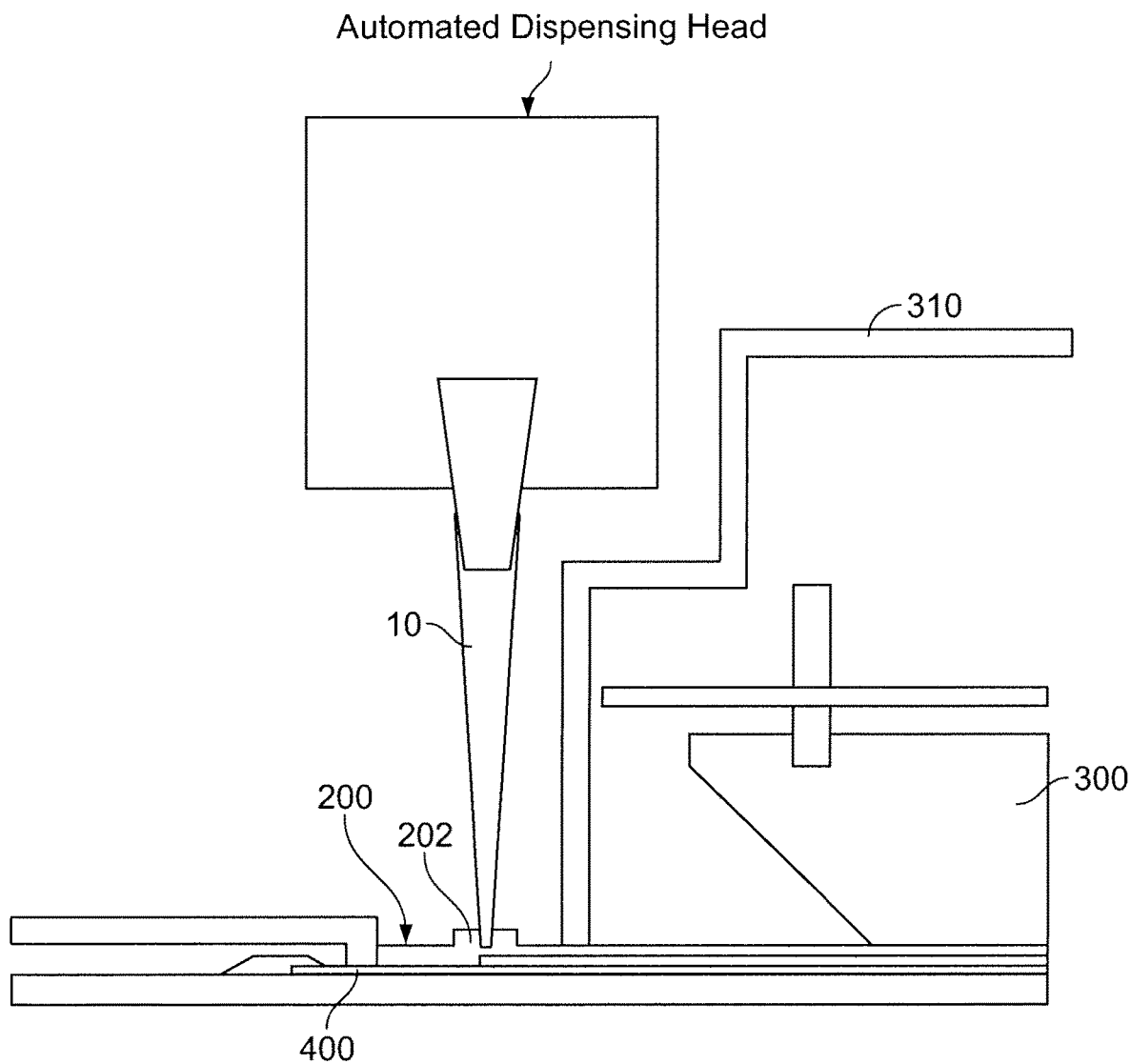


FIG. 35

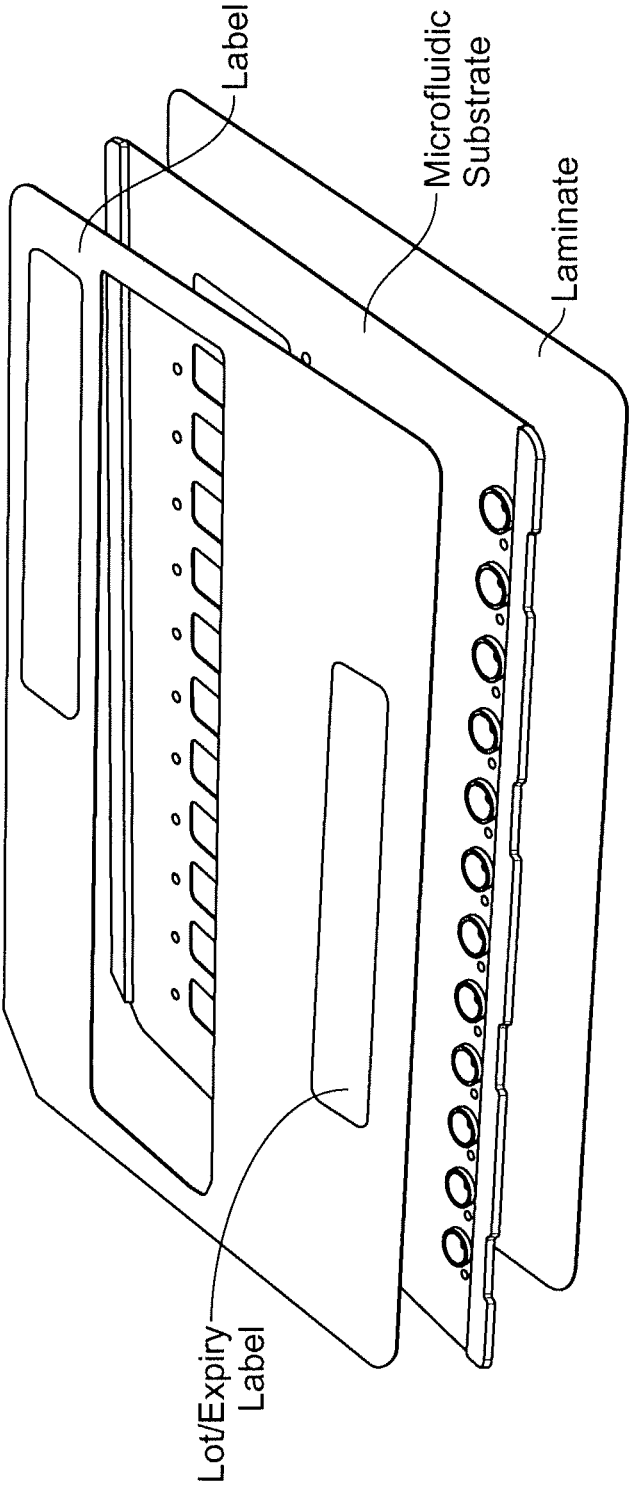


FIG. 36

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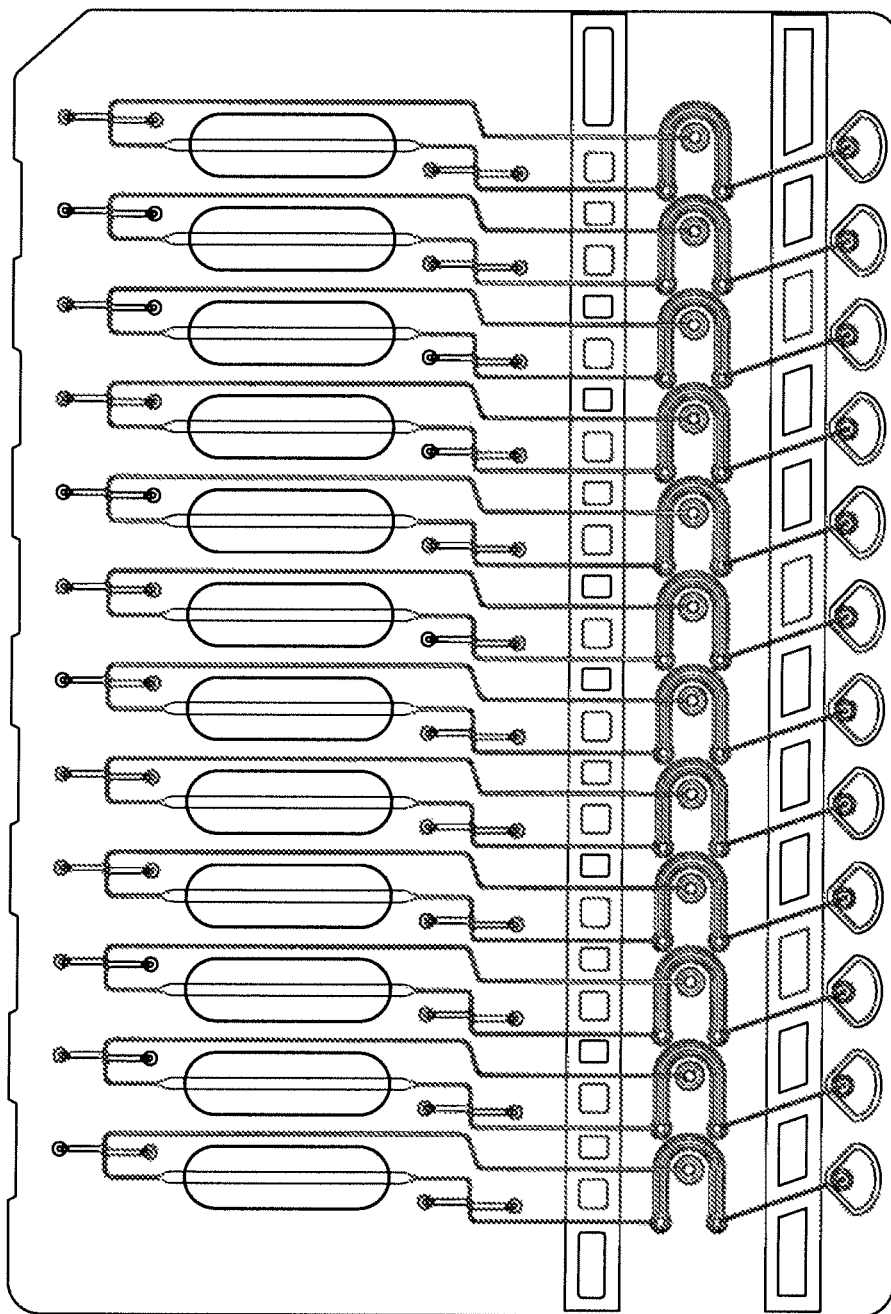


FIG. 37

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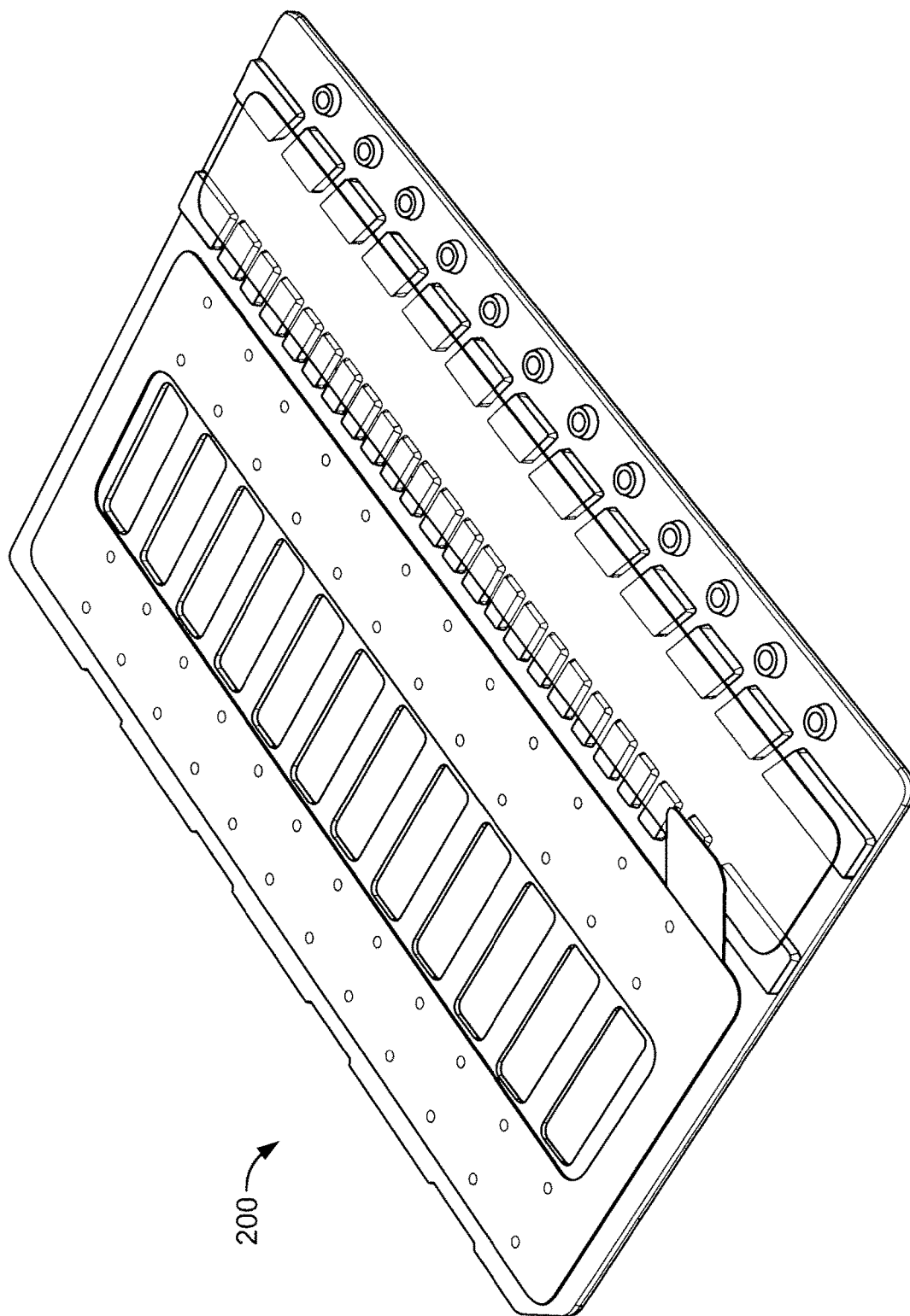


FIG. 38A

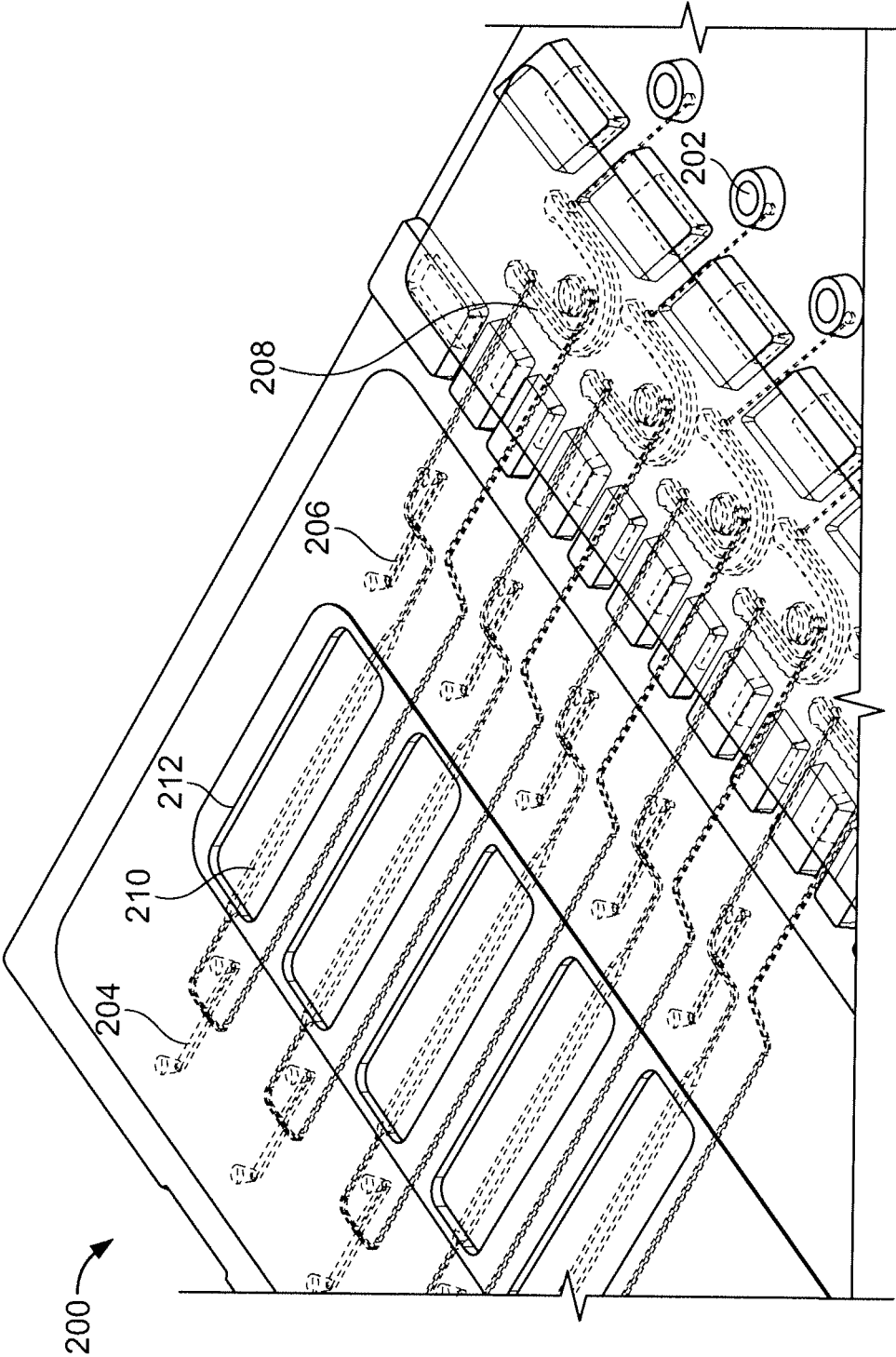


FIG. 38B

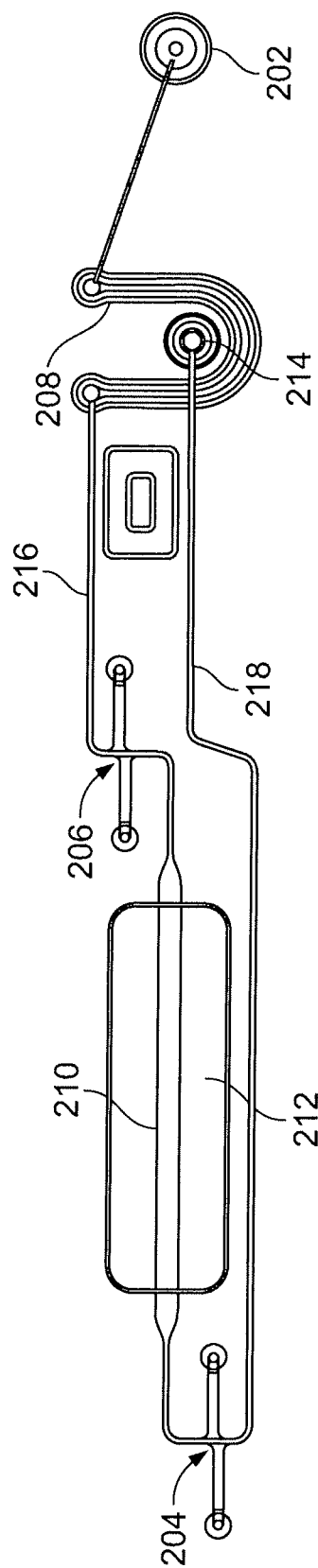


FIG. 39A

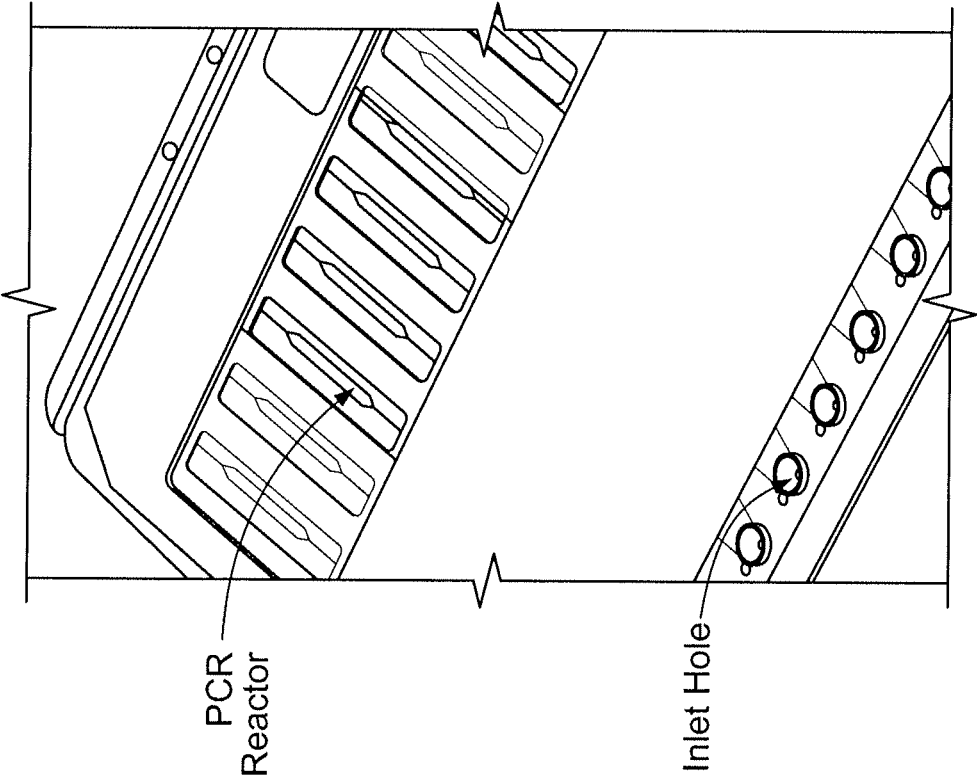
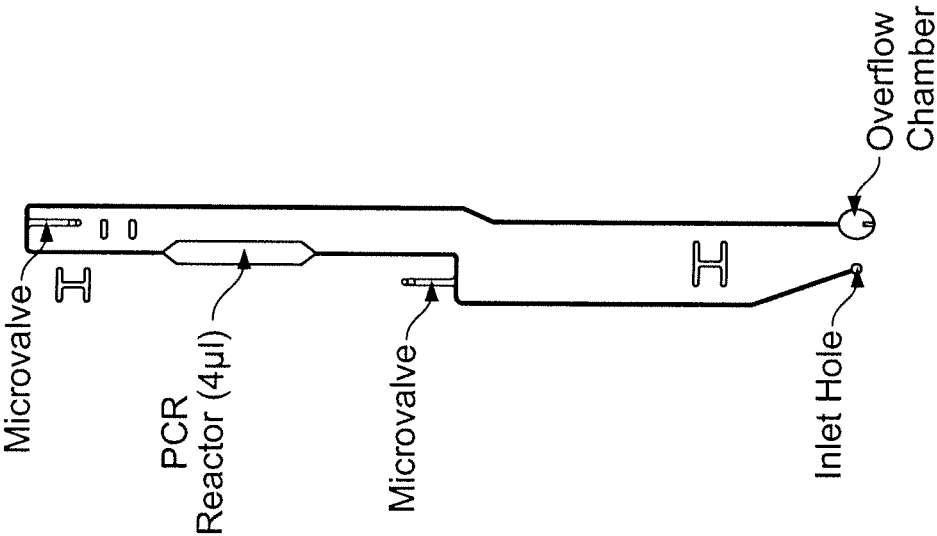


FIG. 39B



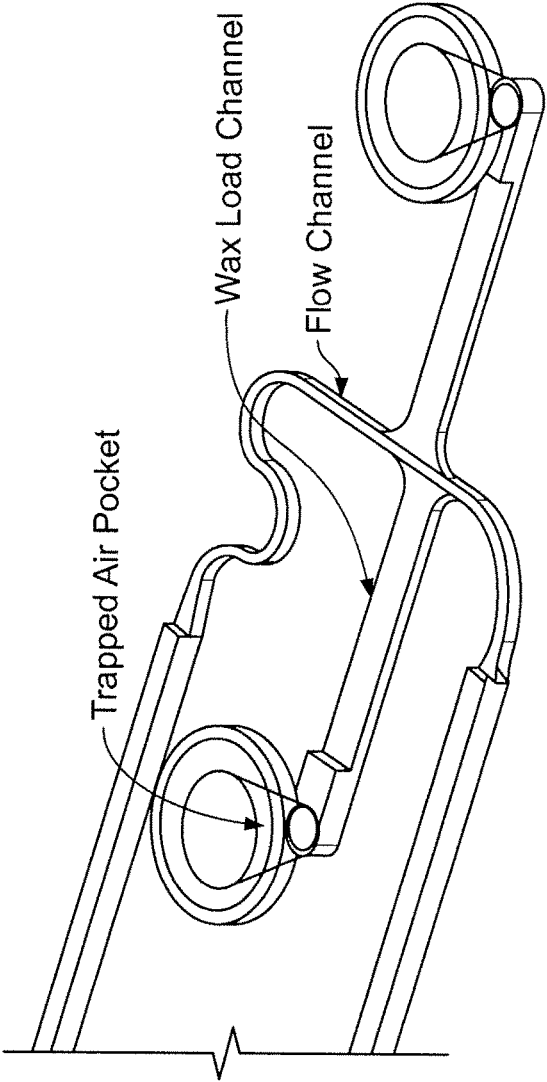
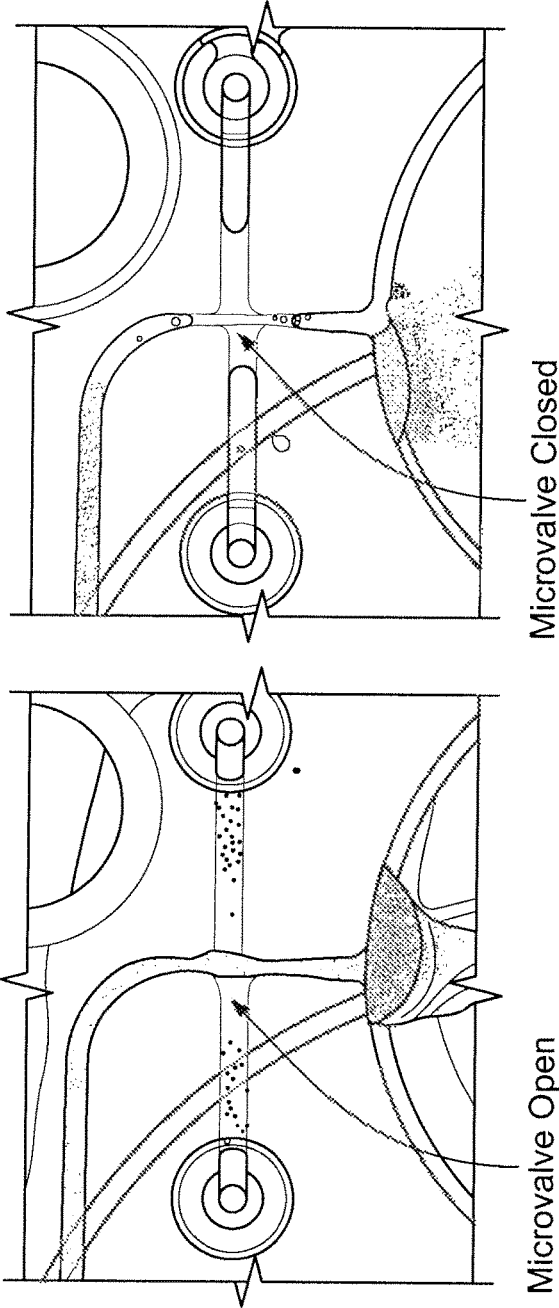


FIG. 40A

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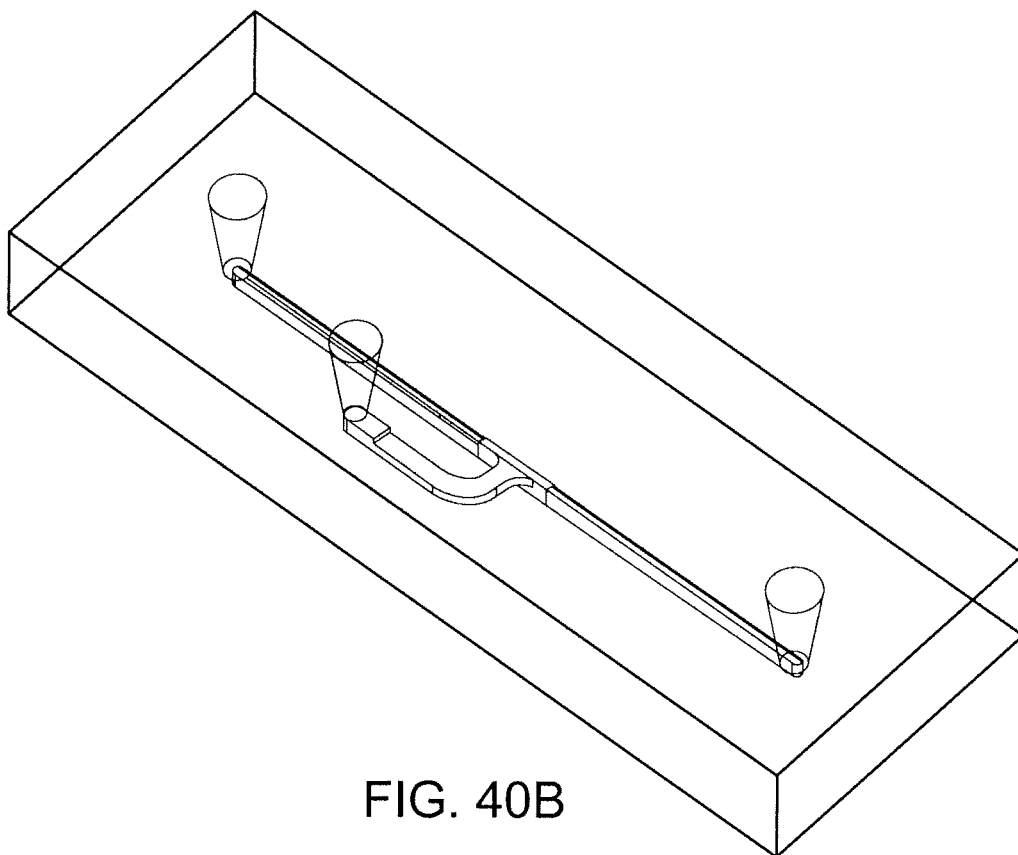


FIG. 40B

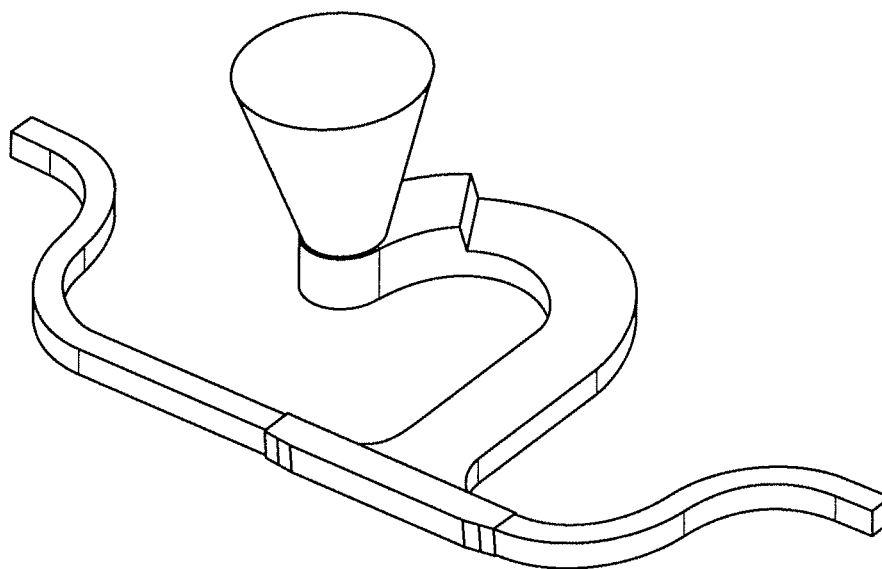


FIG. 40C

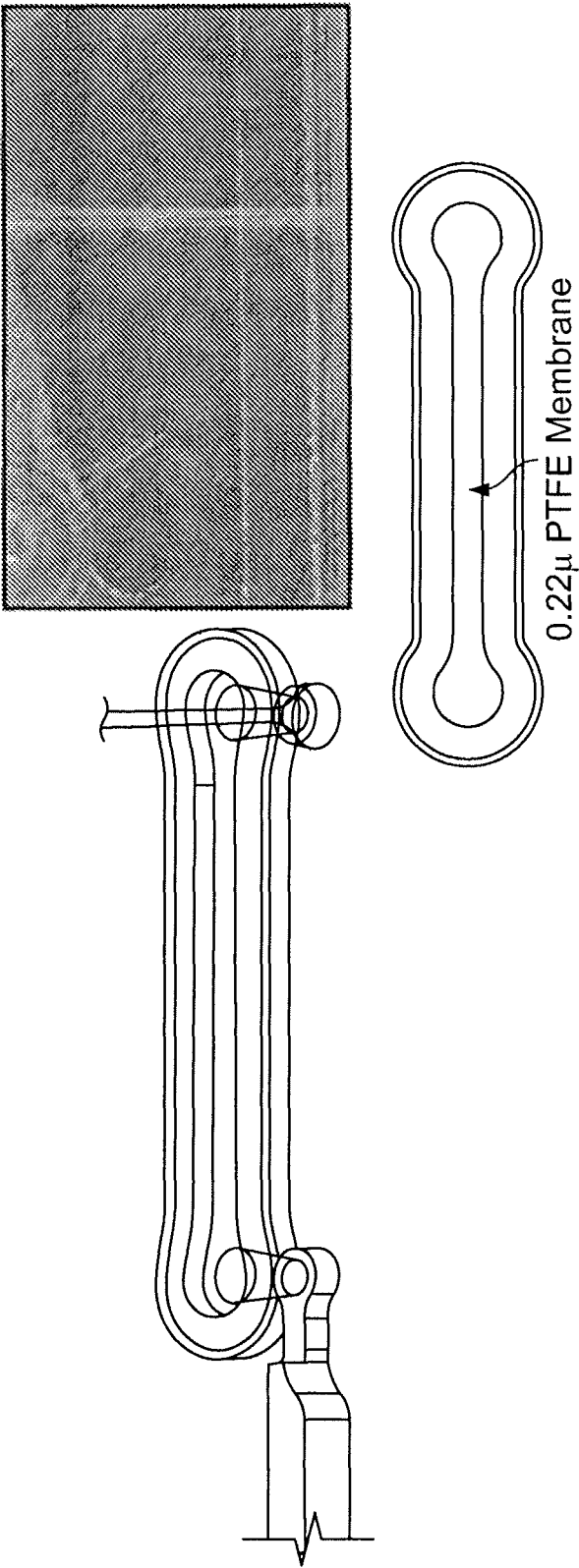


FIG. 41

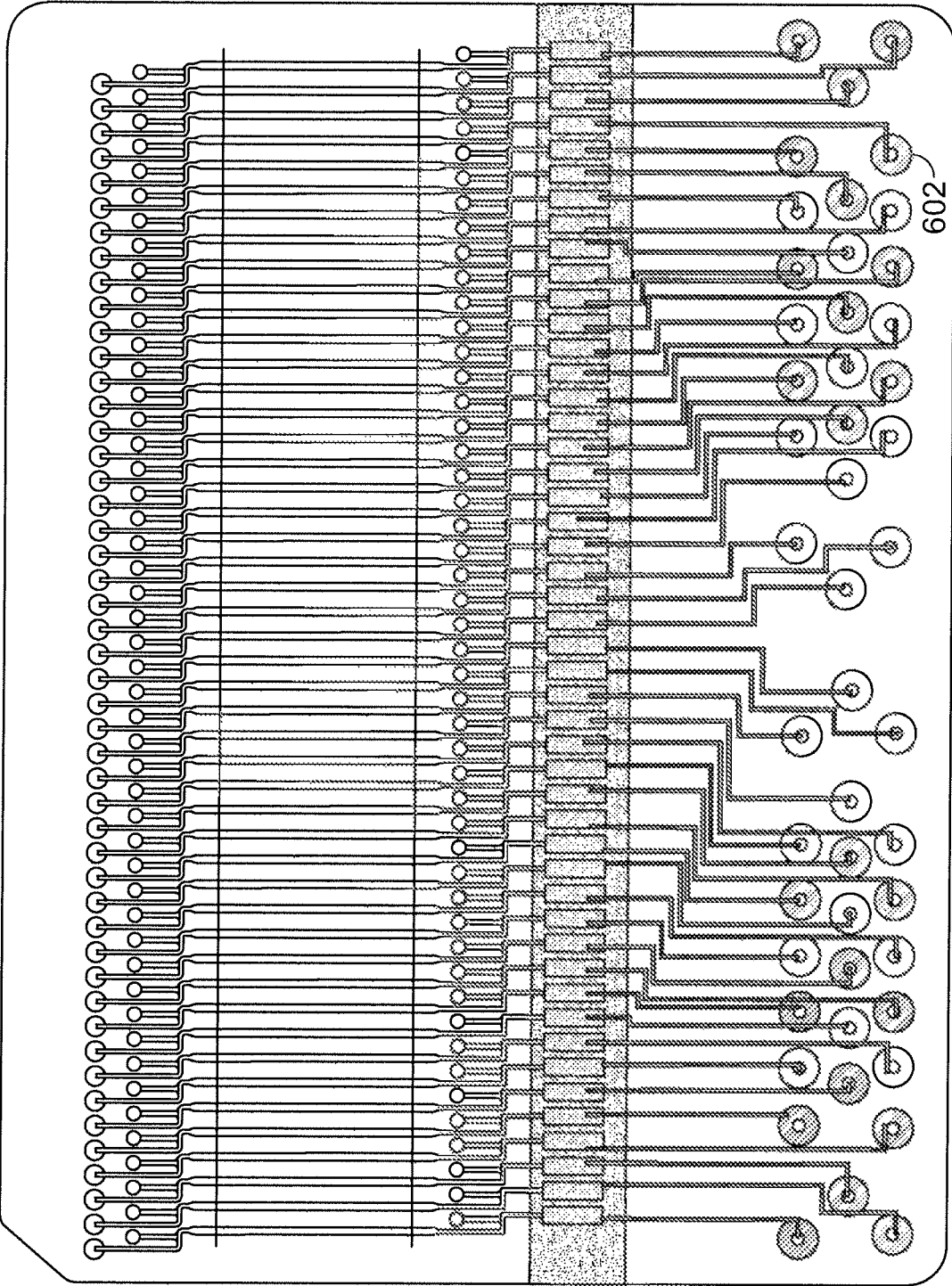


FIG 42

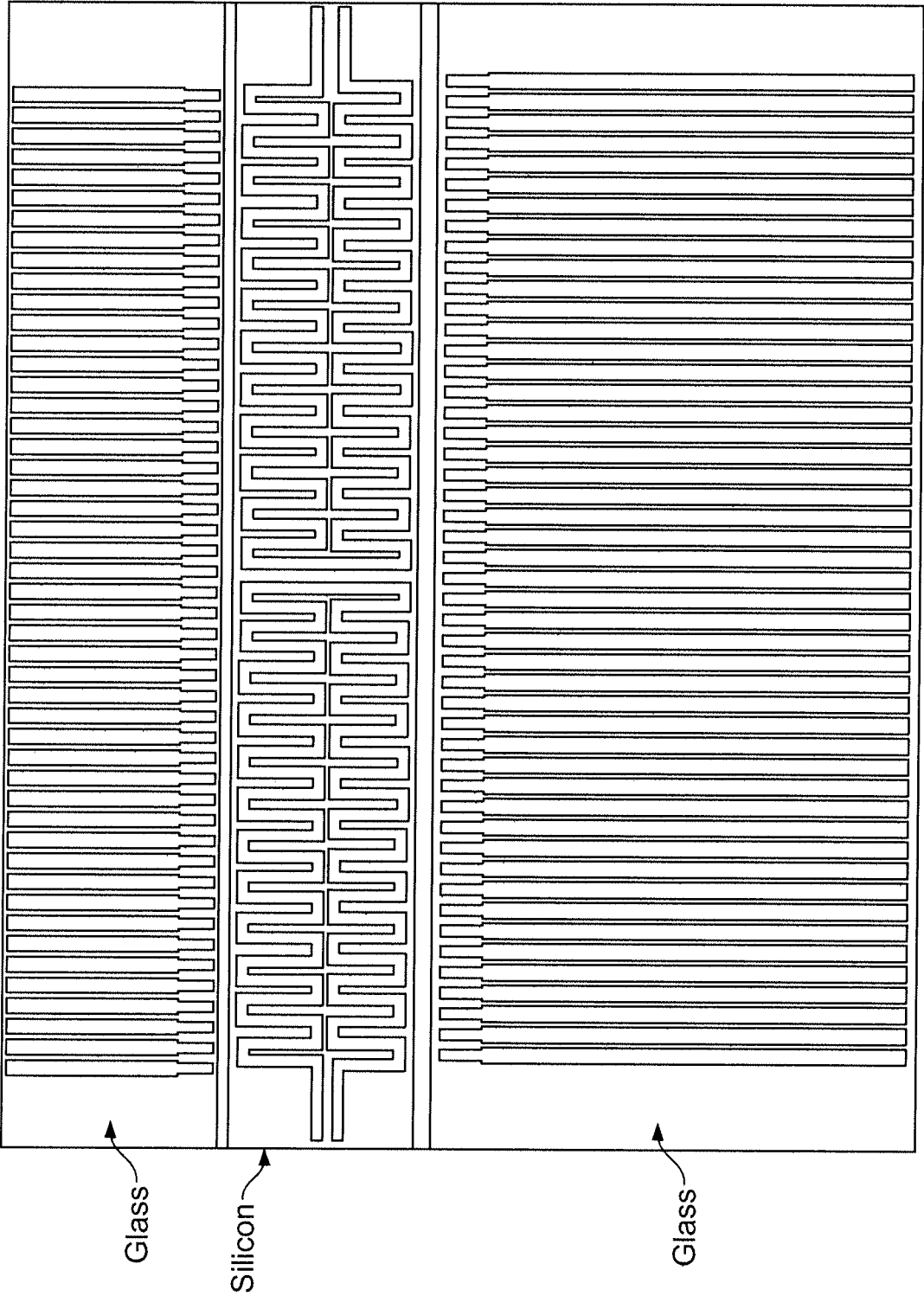


FIG. 43

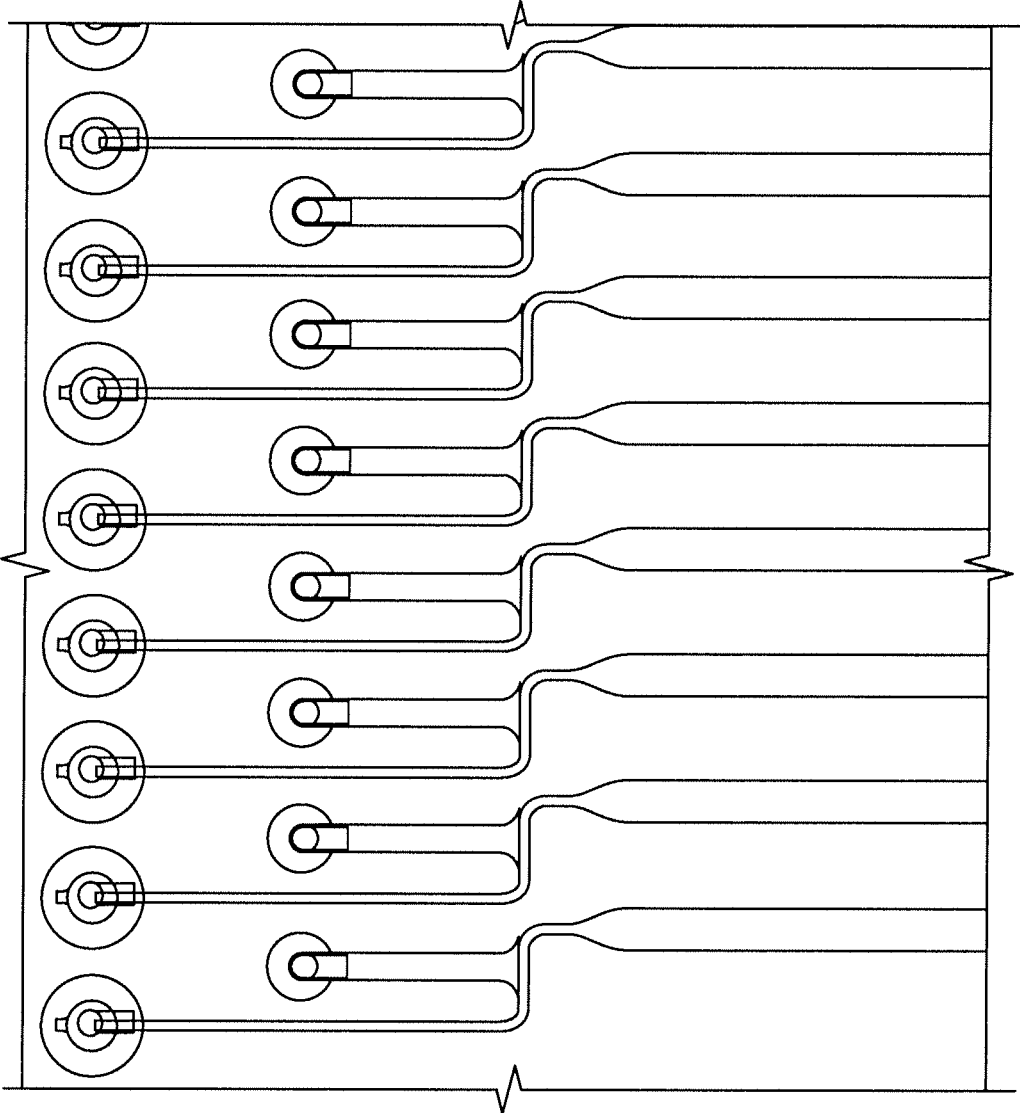


FIG. 44

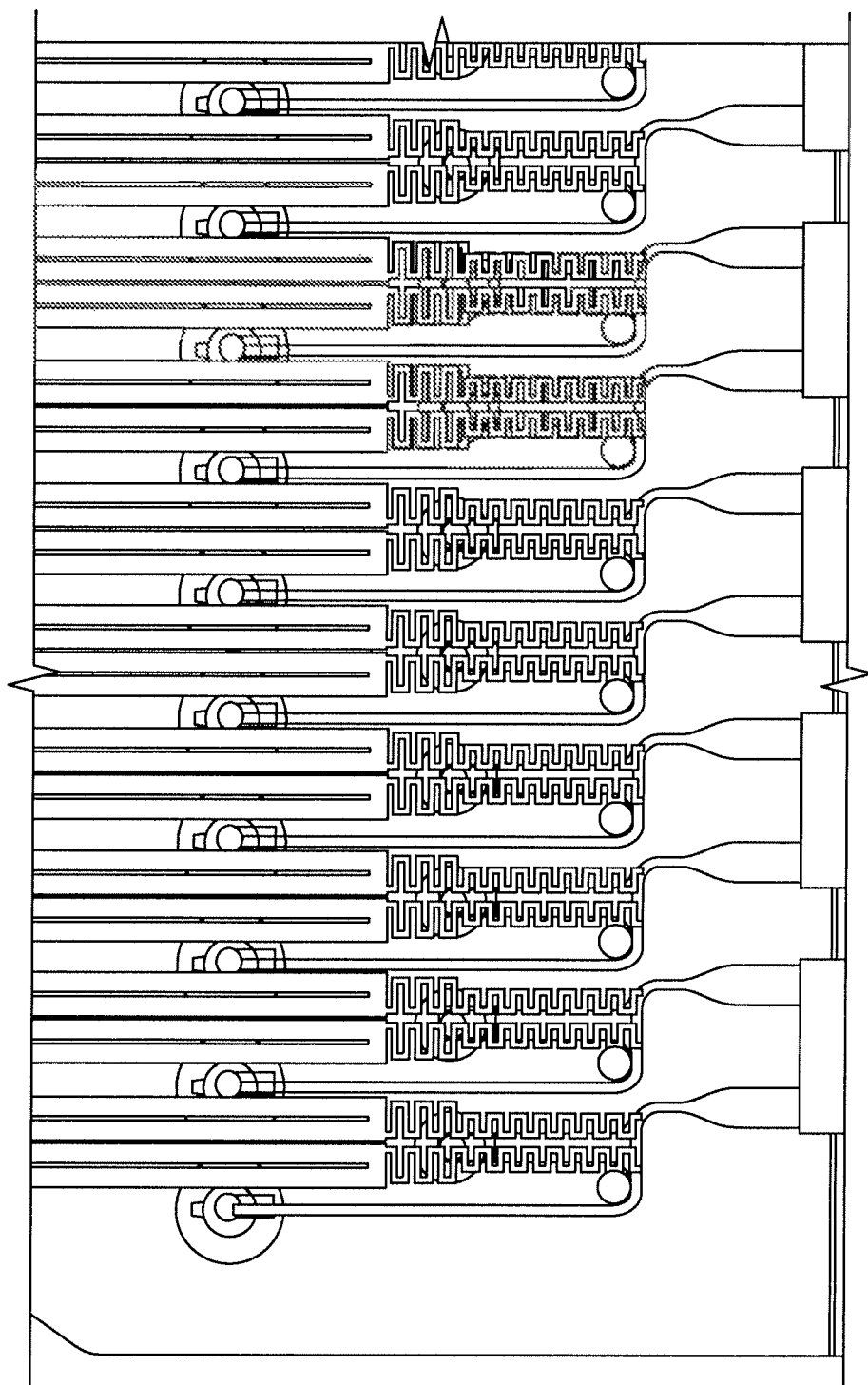


FIG. 45

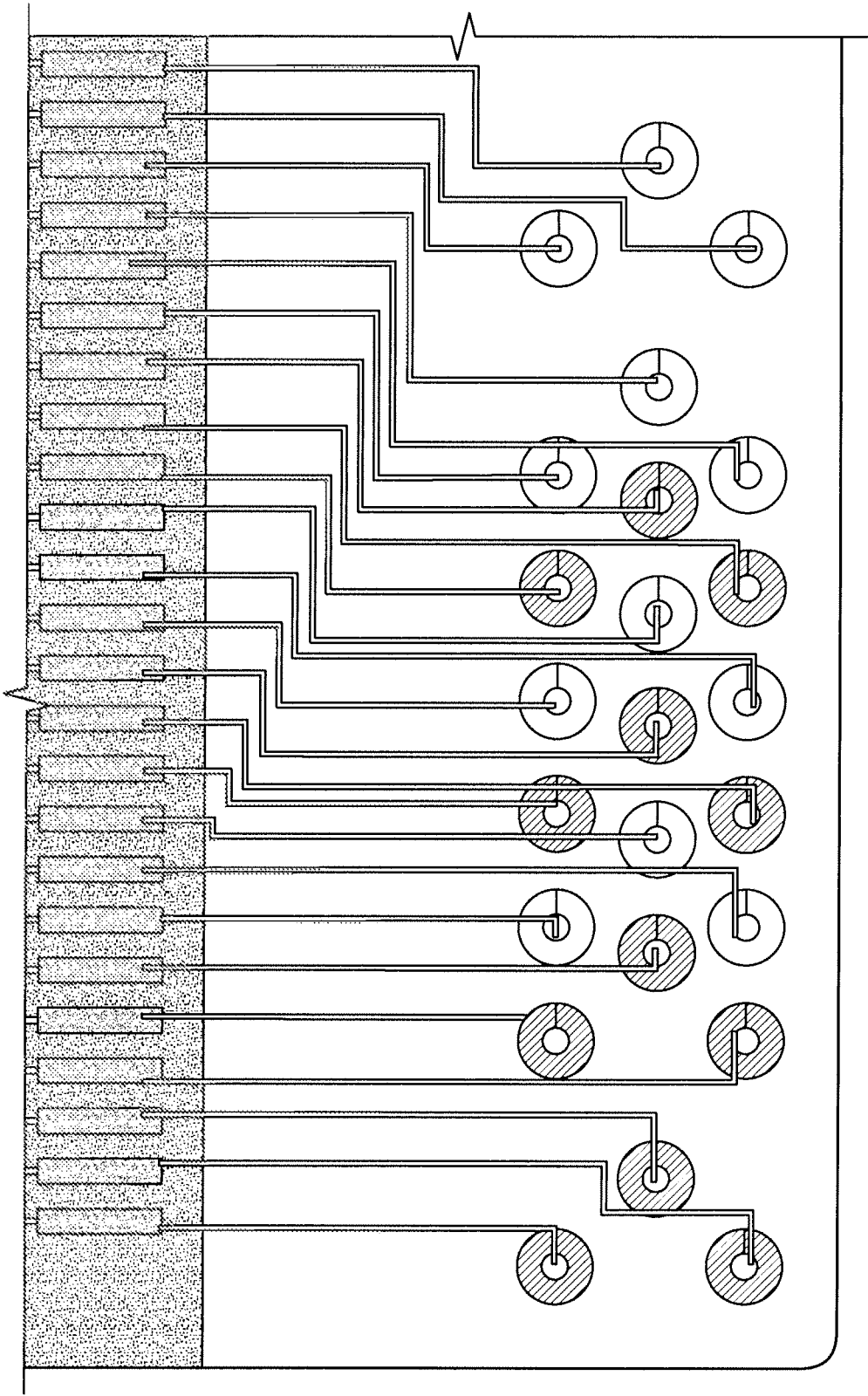


FIG. 46

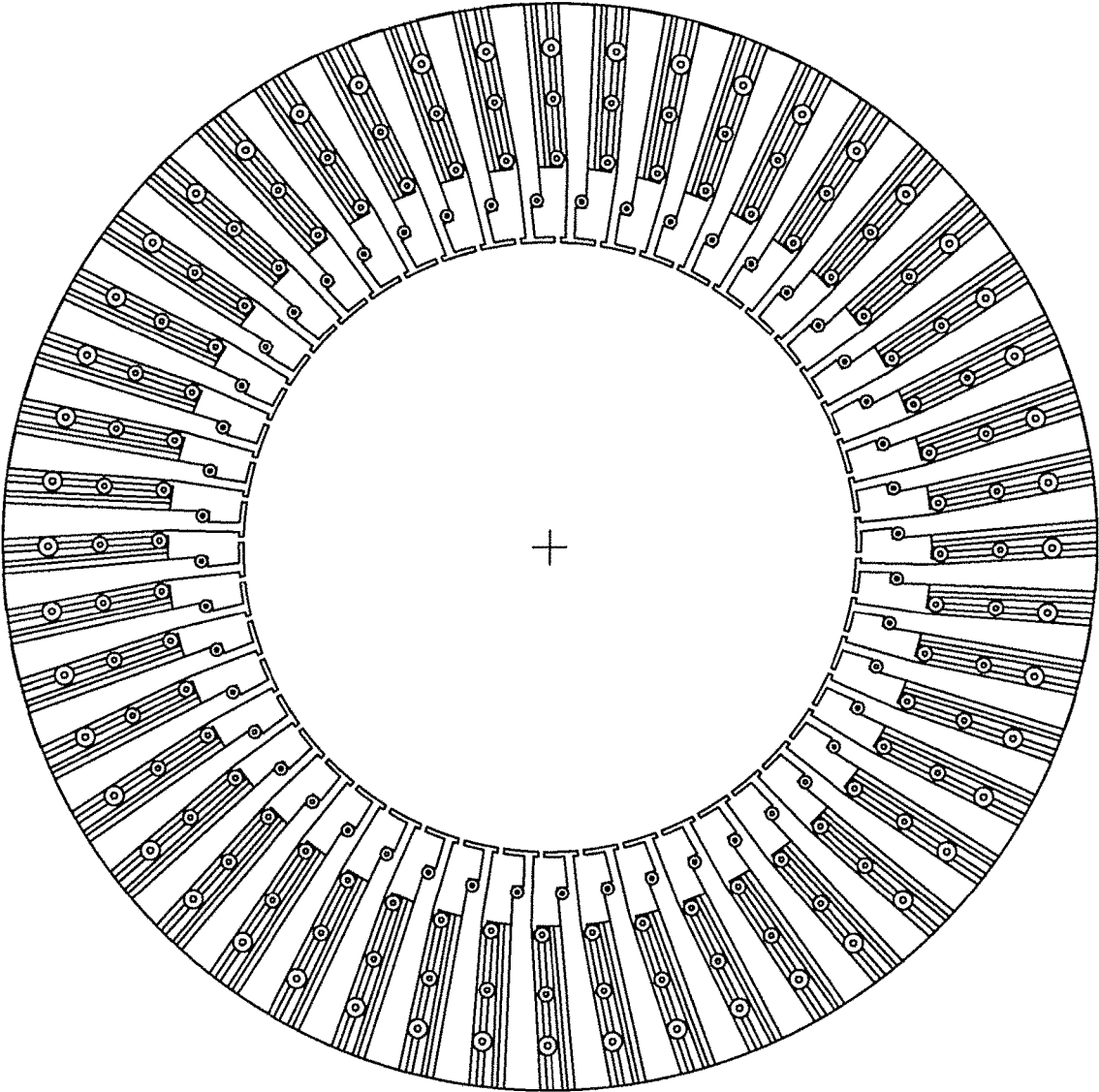


FIG. 47A

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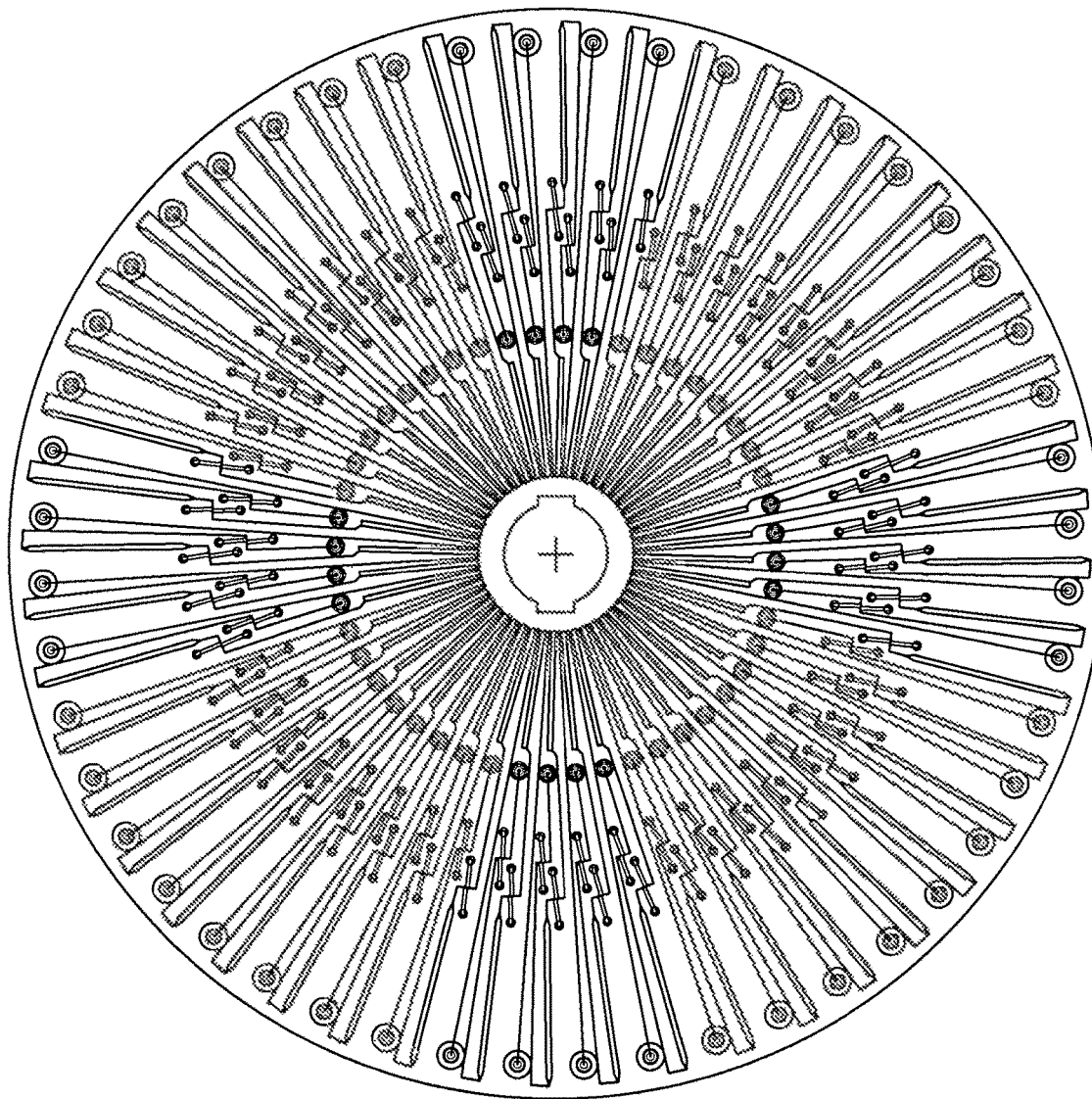


FIG. 47B

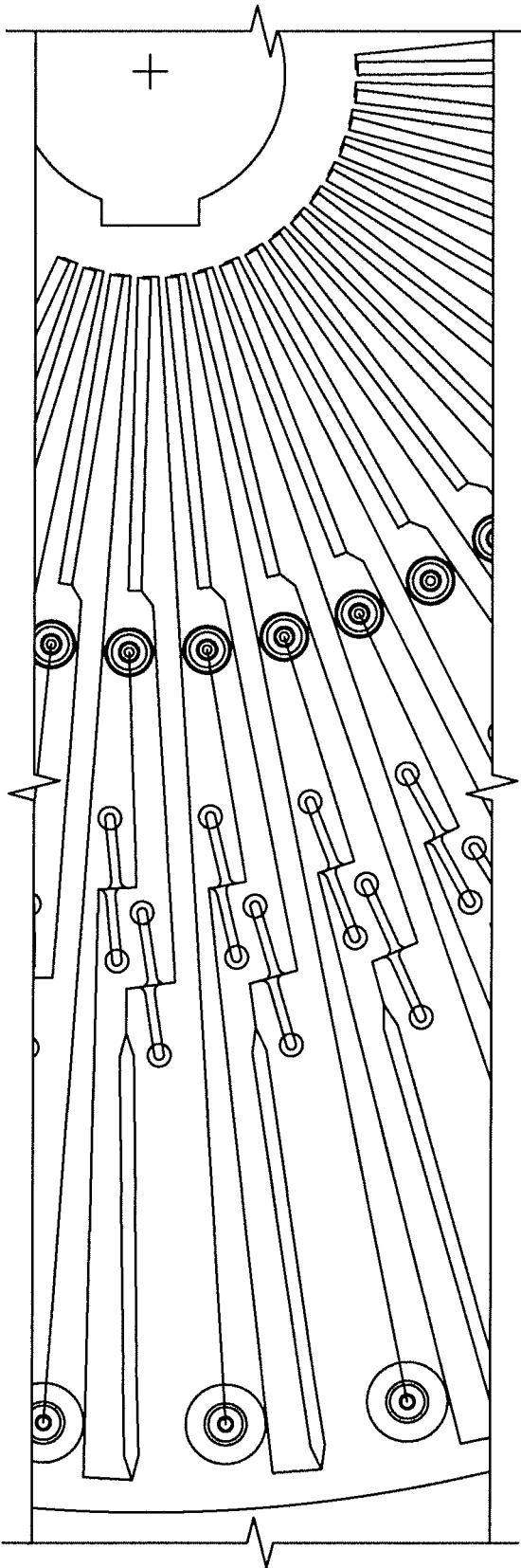


FIG. 47C

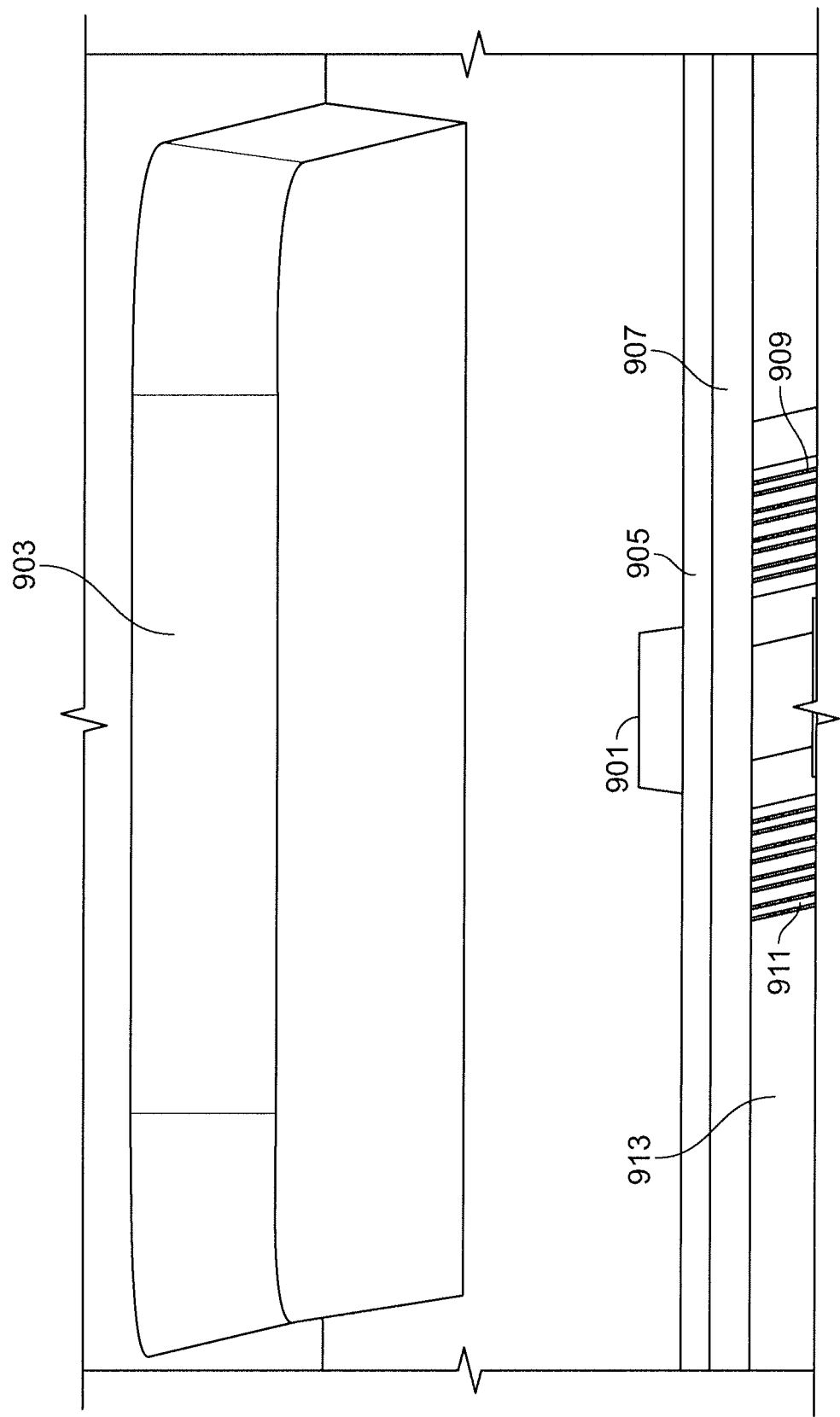


FIG. 48

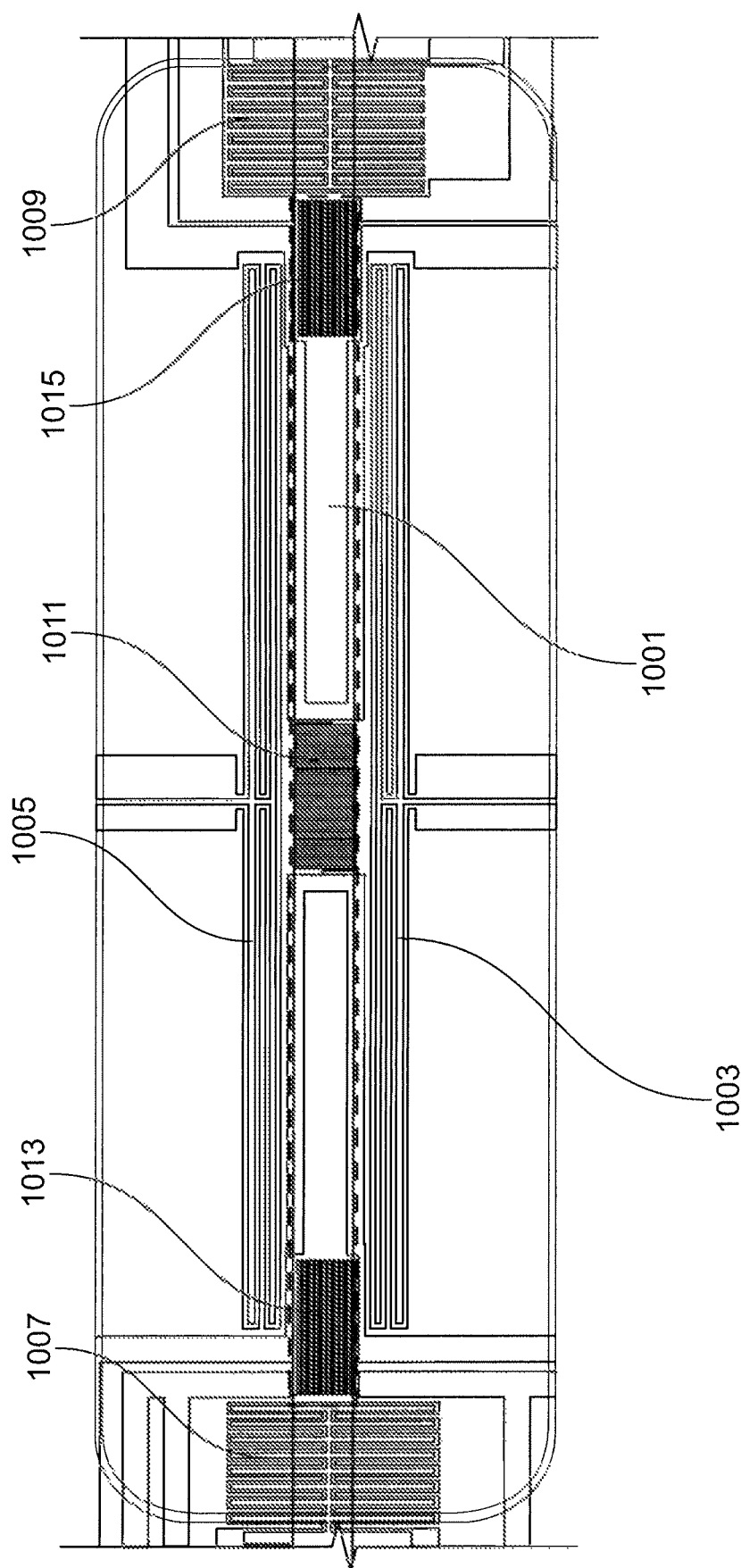


FIG. 49A

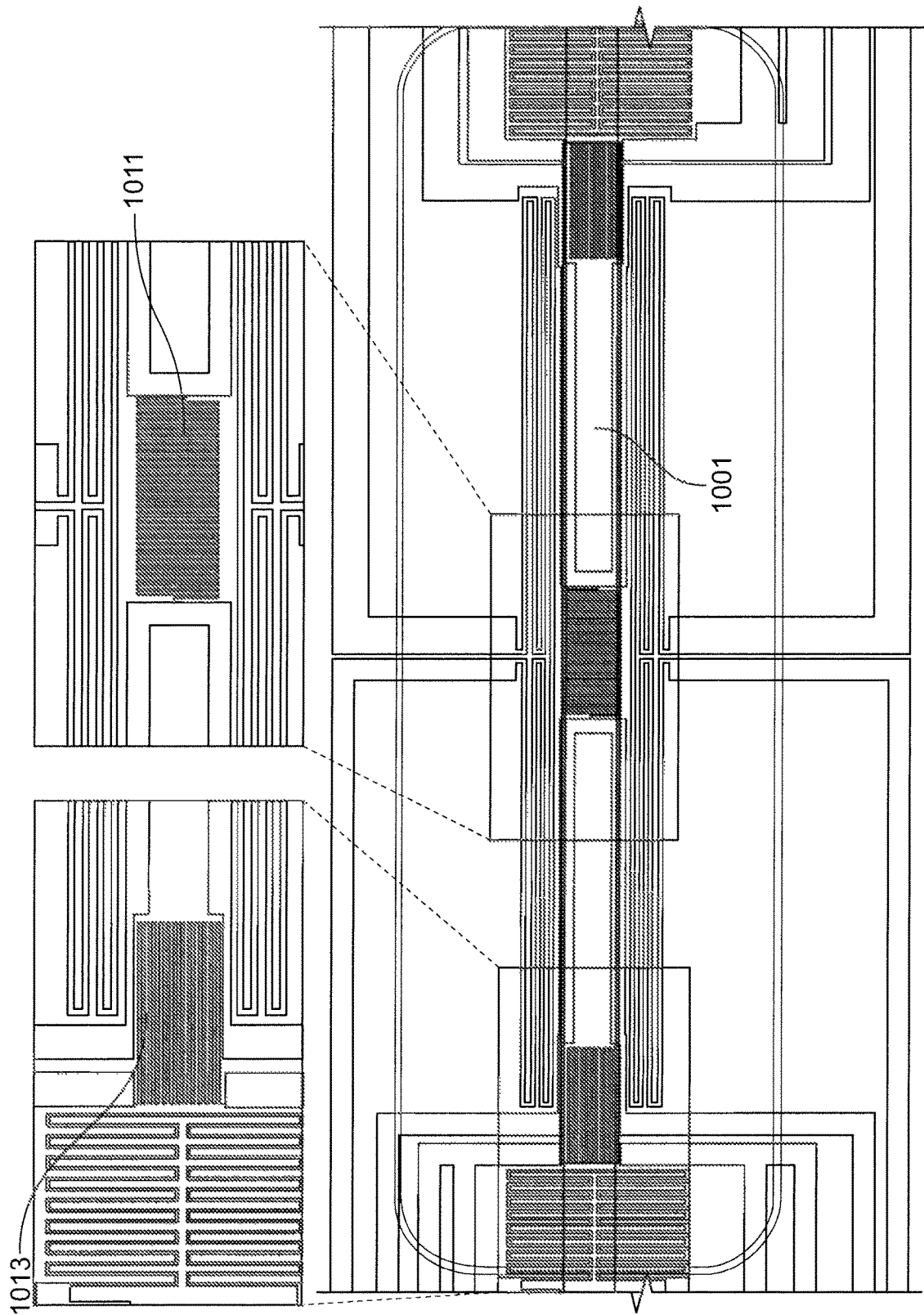


FIG. 49B

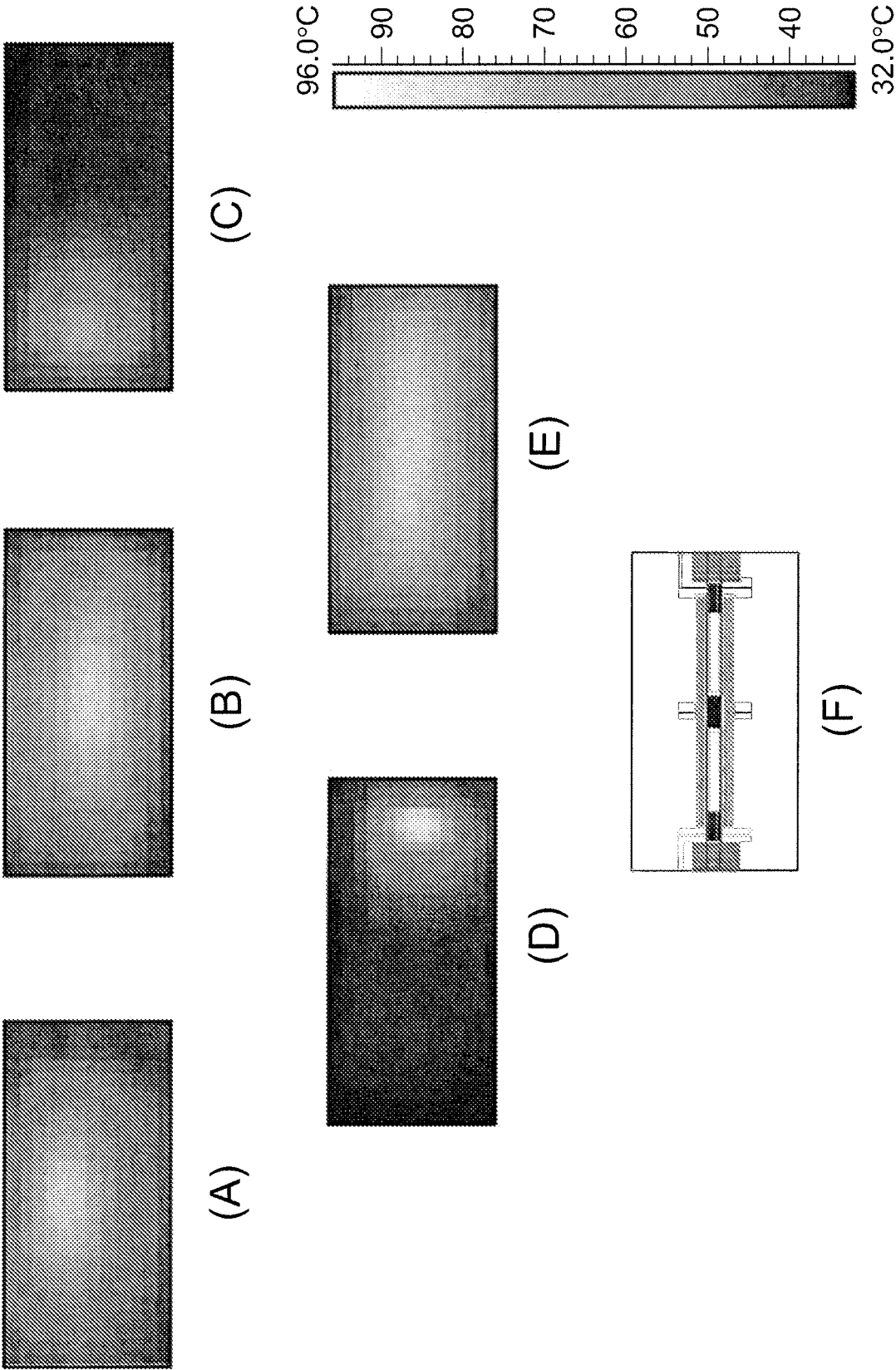
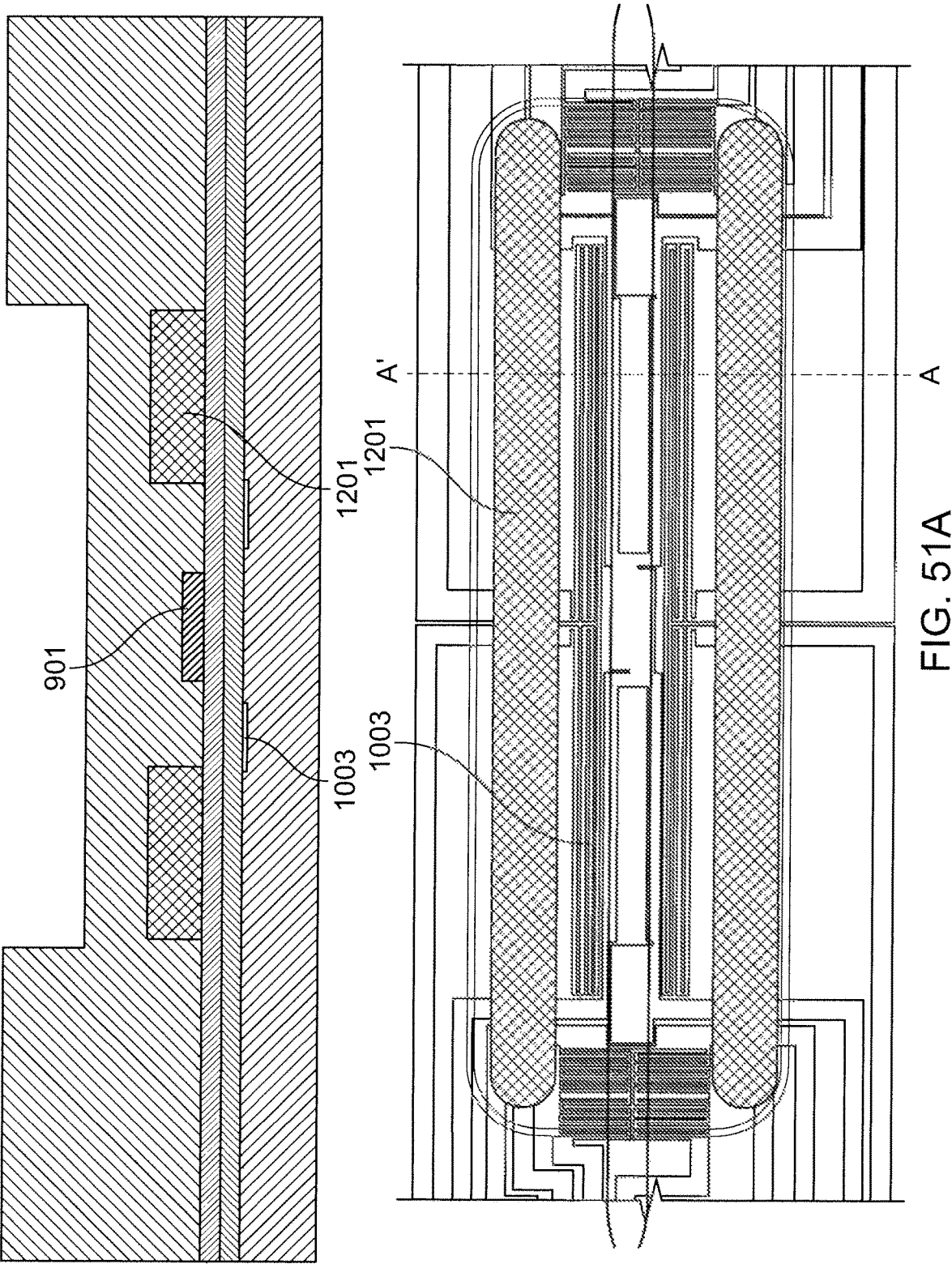


FIG. 50



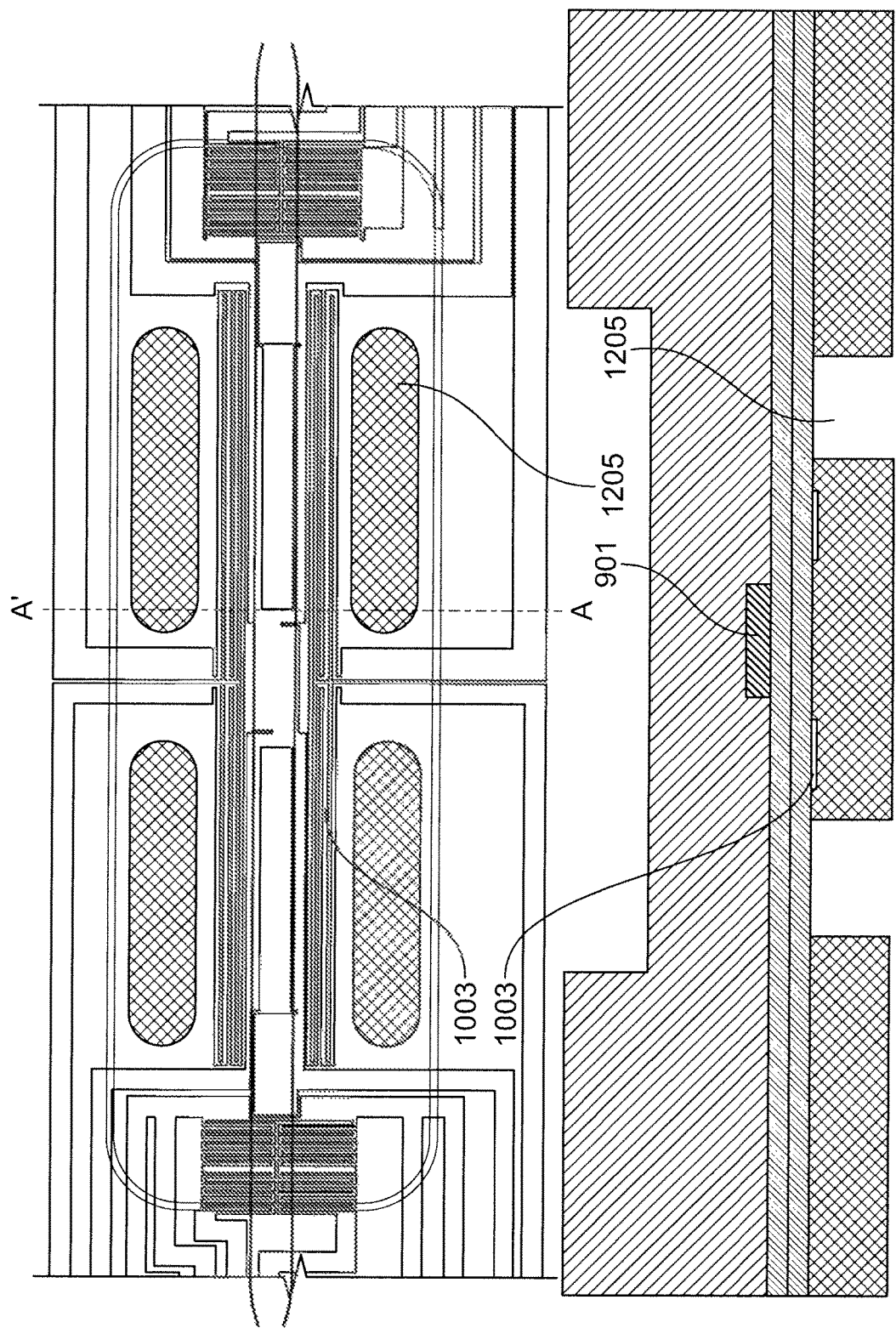


FIG. 51B

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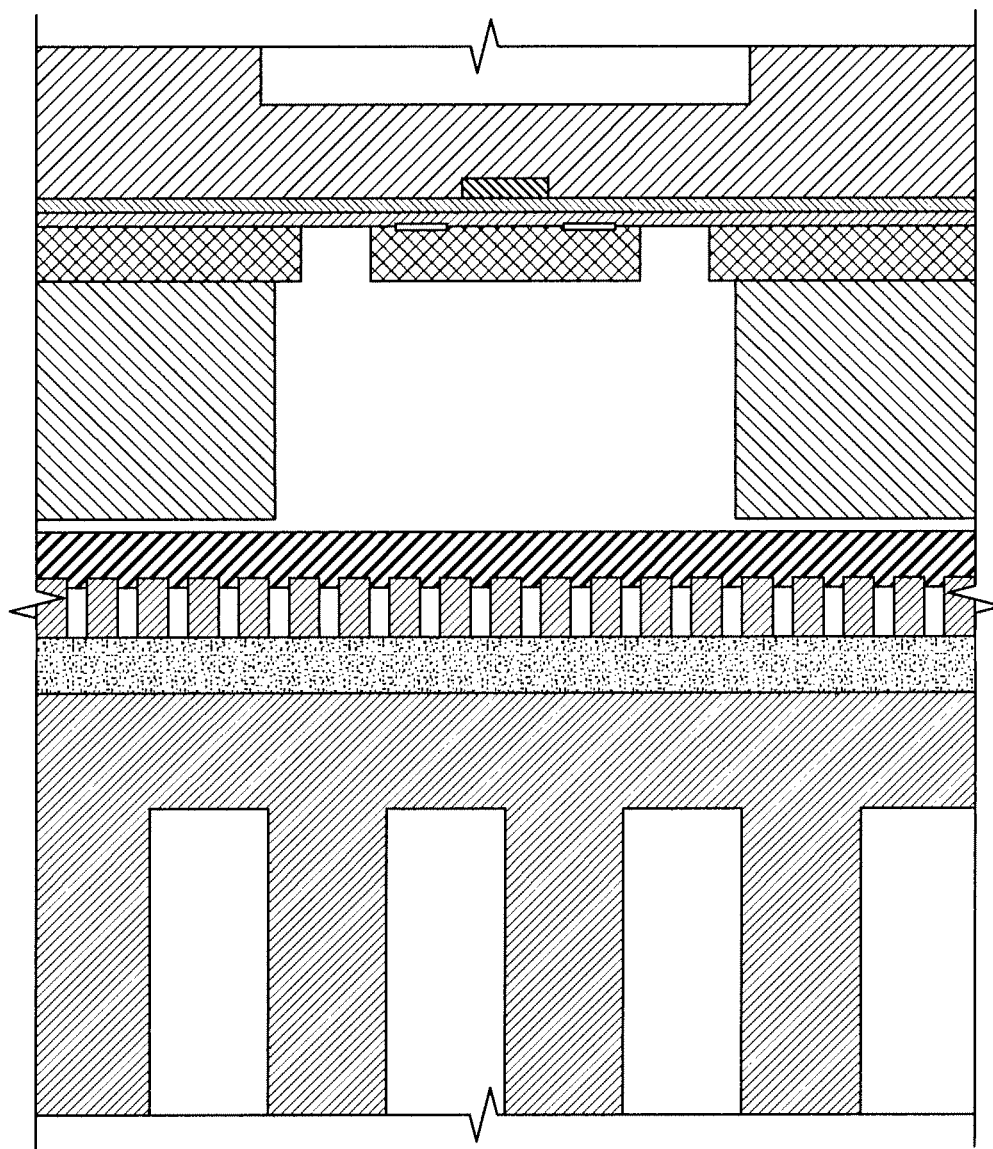


FIG. 51C

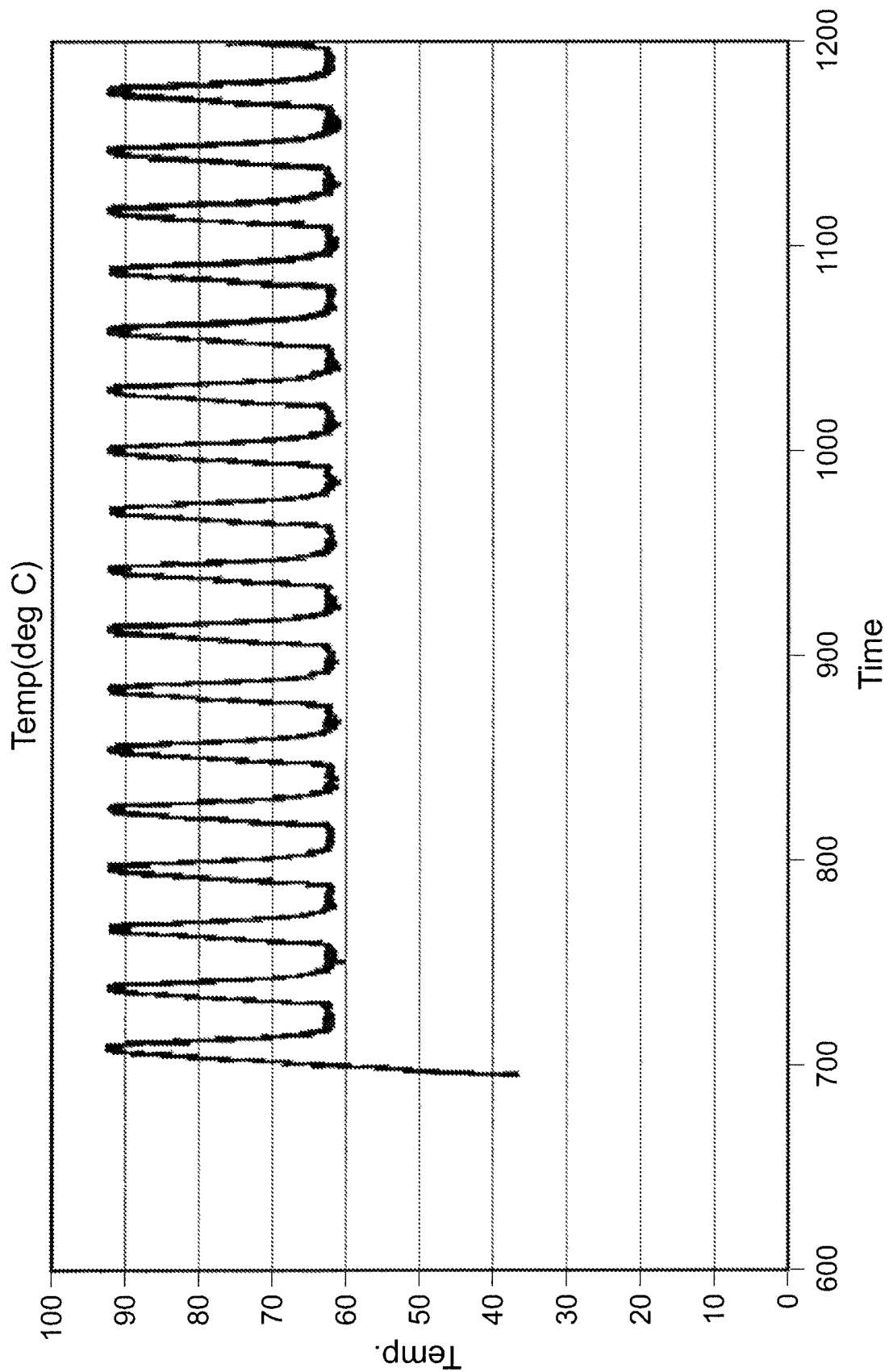


FIG. 52

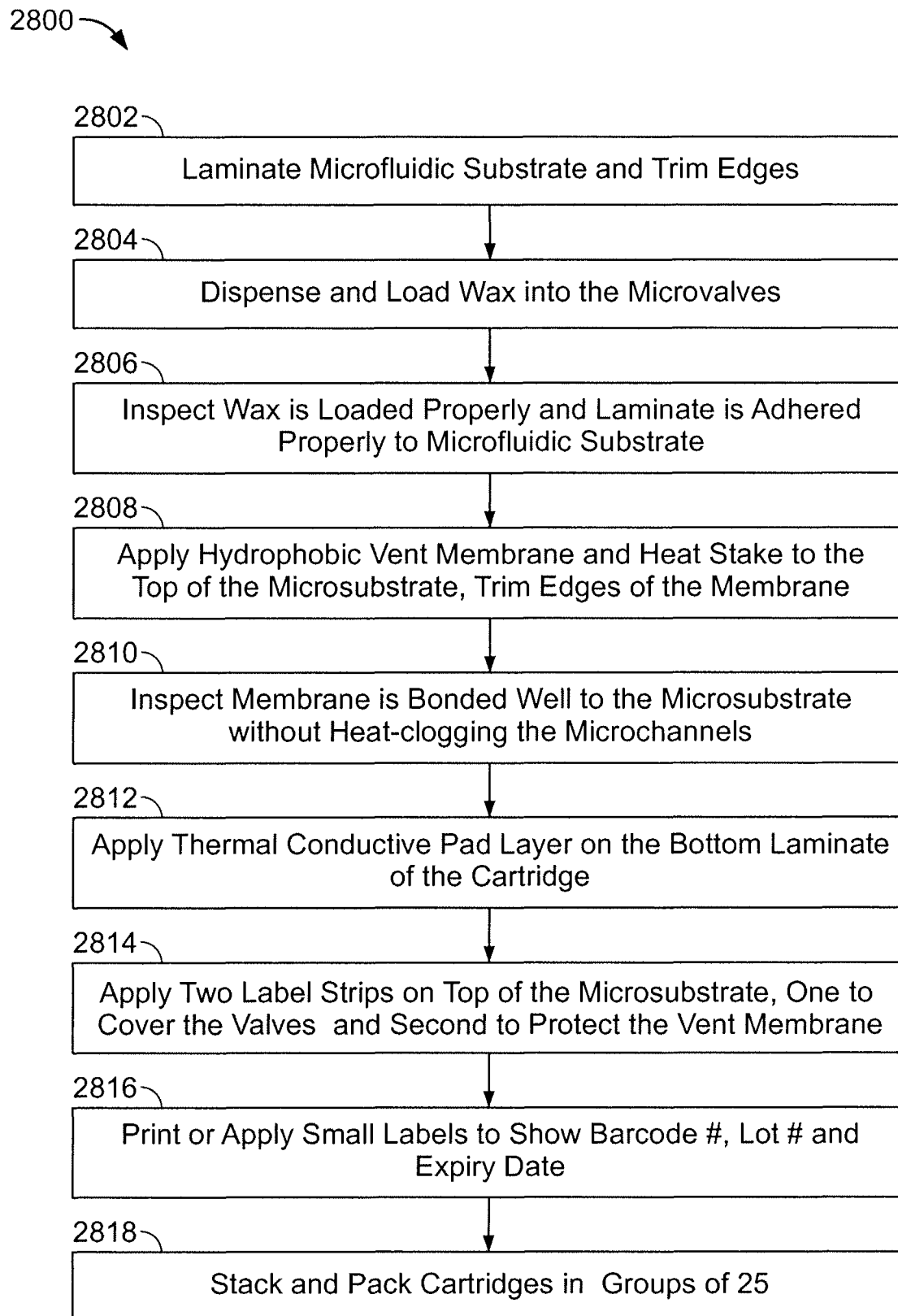


FIG. 53

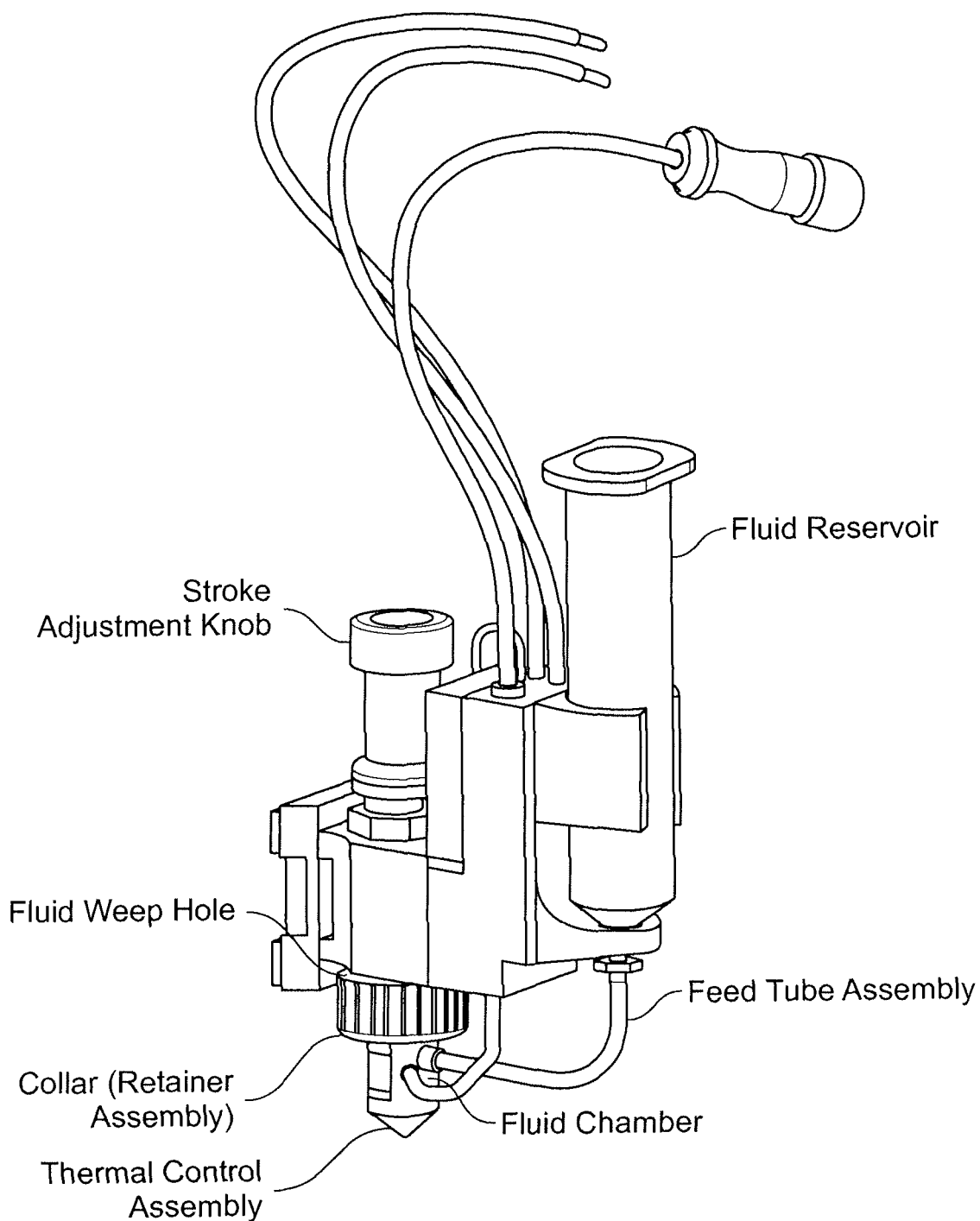


FIG. 54A

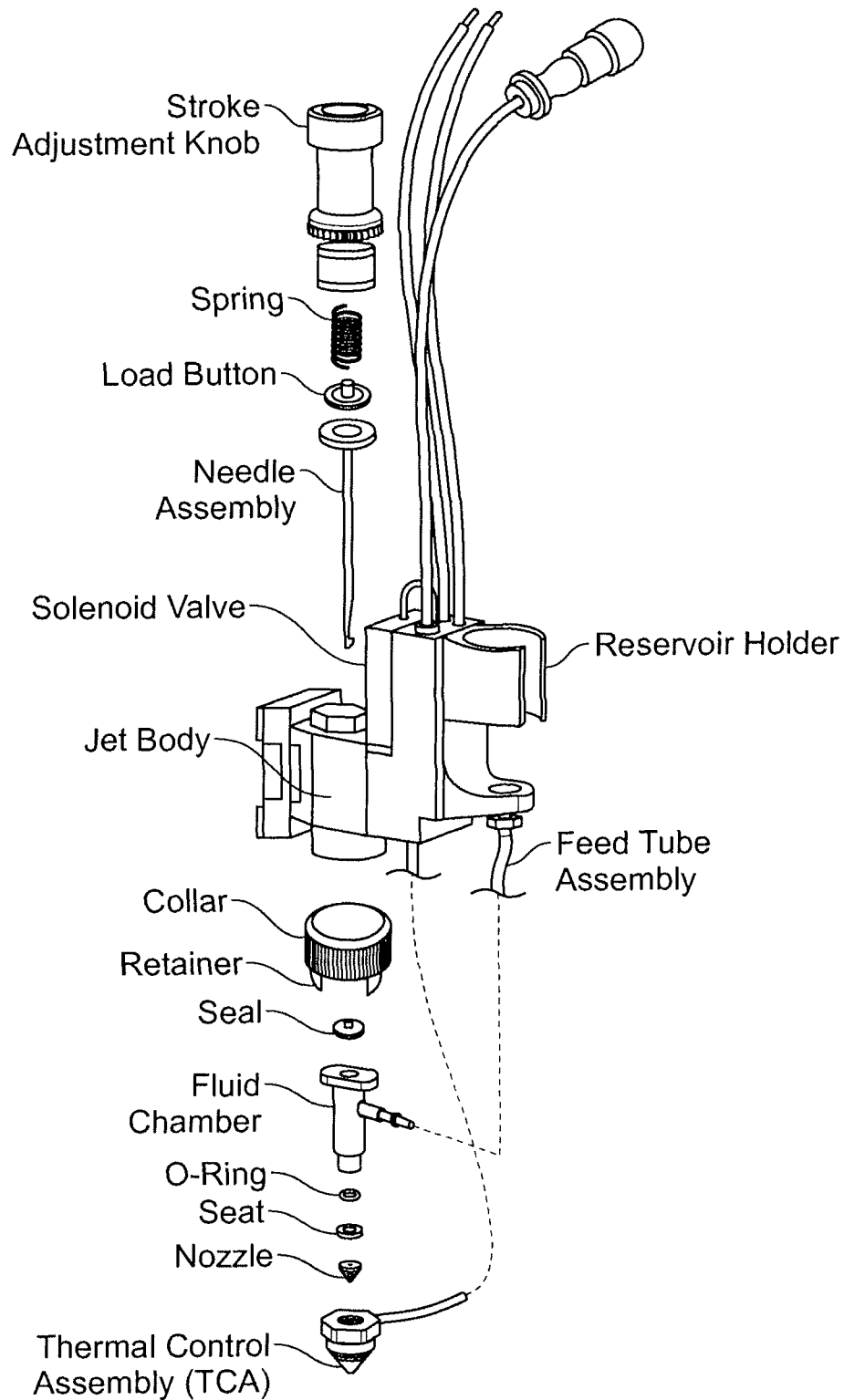


FIG. 54B

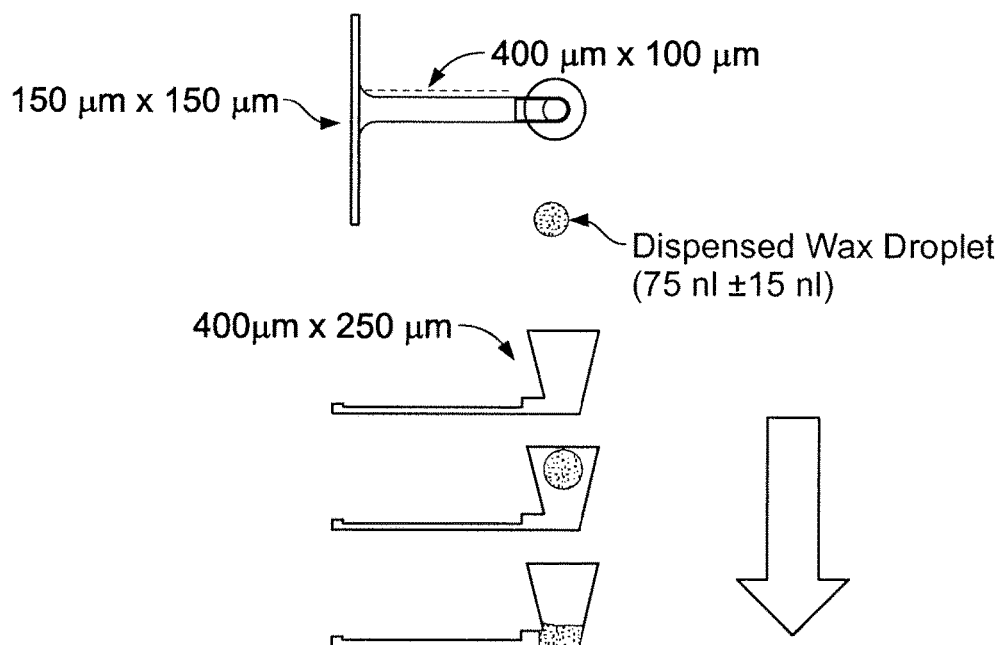


FIG. 55A

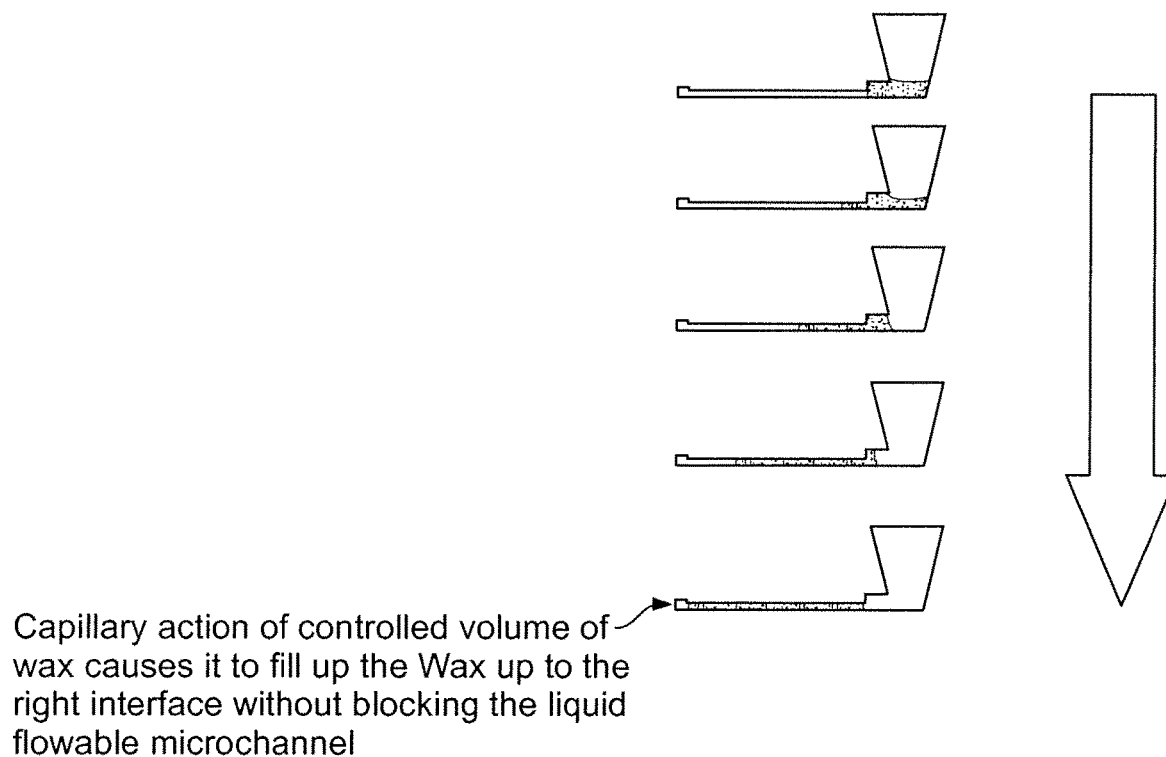


FIG. 55B

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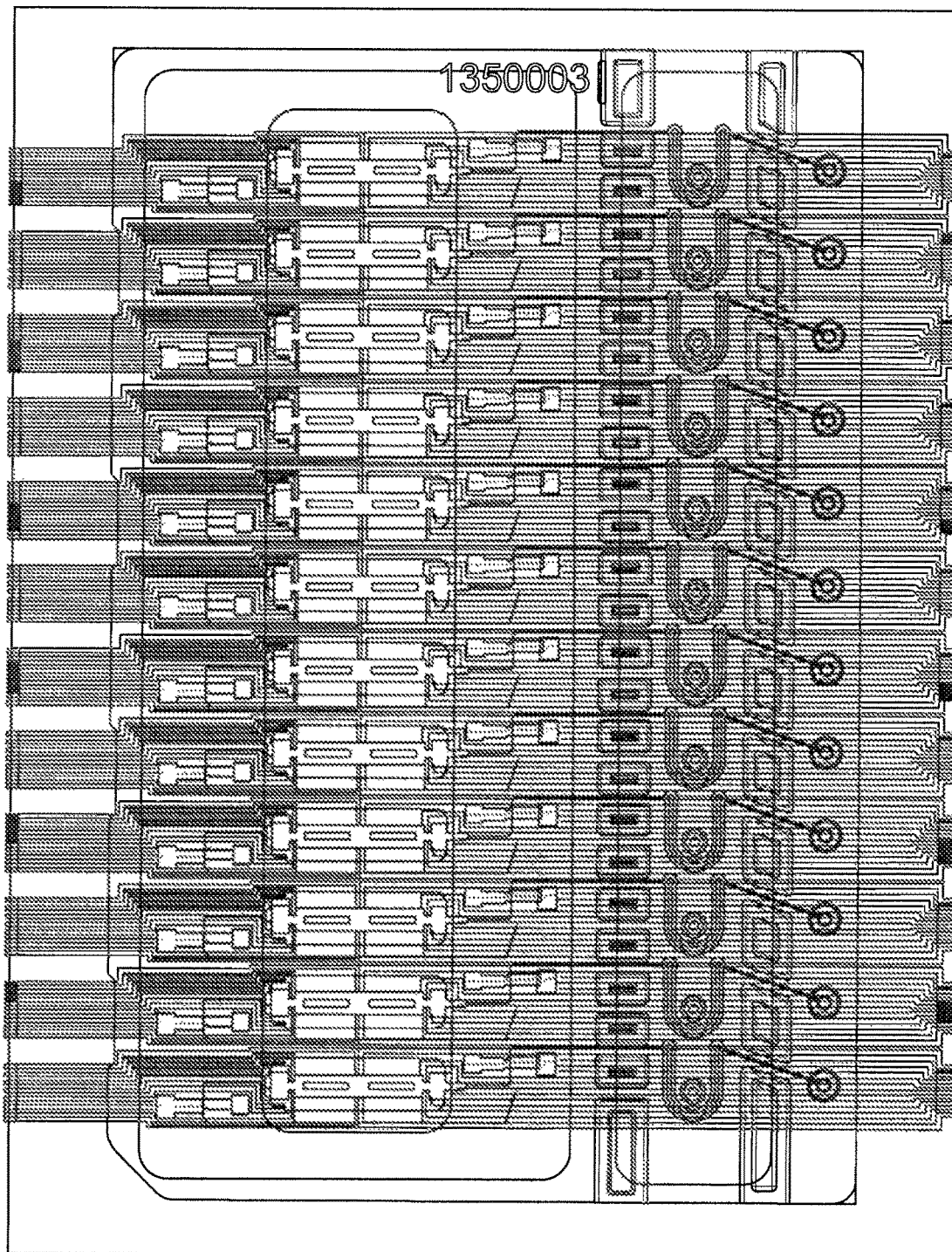


FIG 56

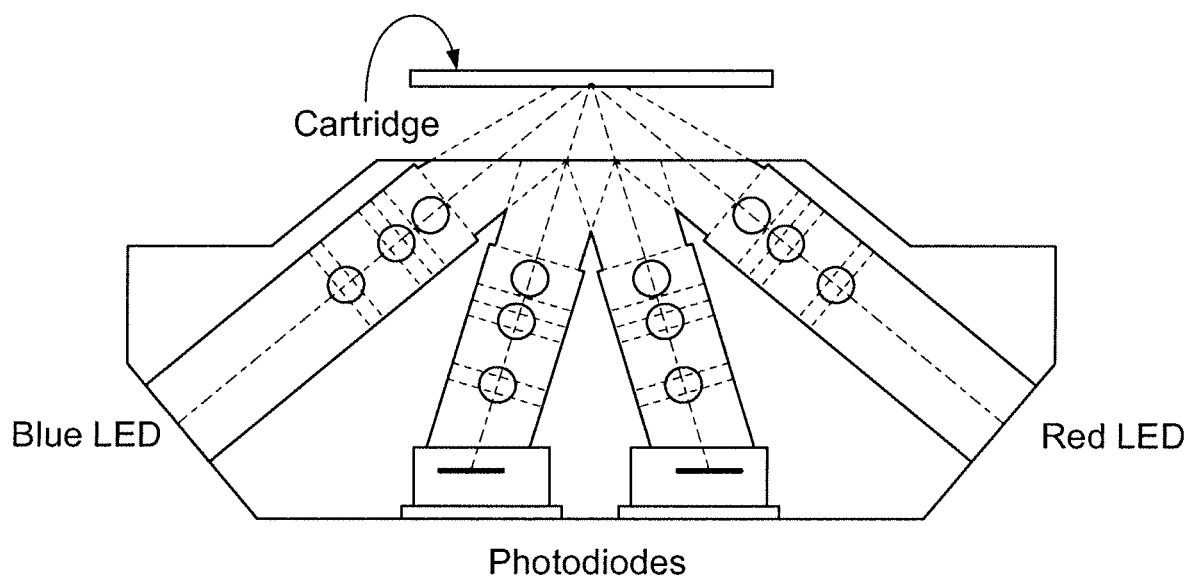


FIG. 57

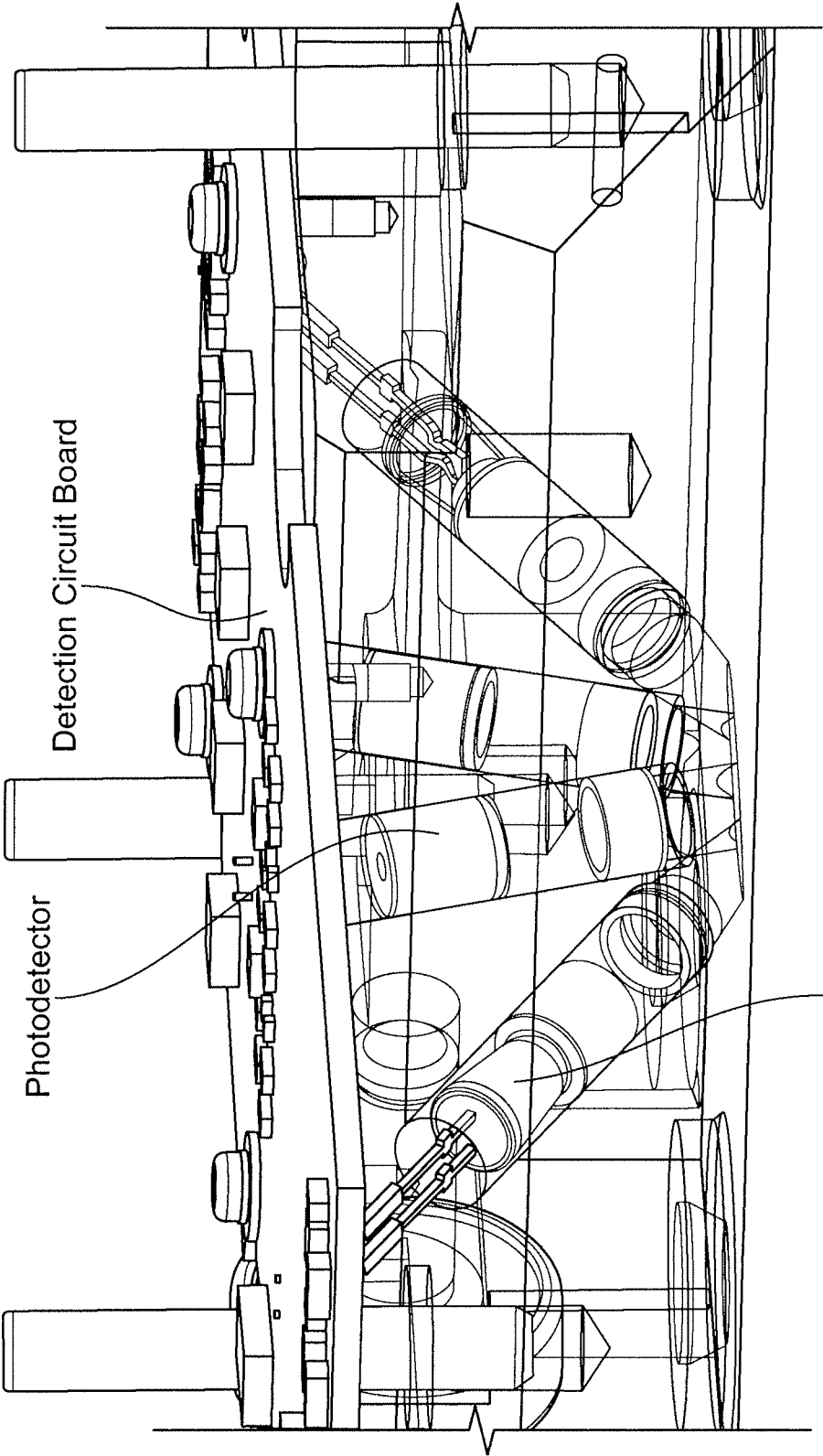


FIG. 58

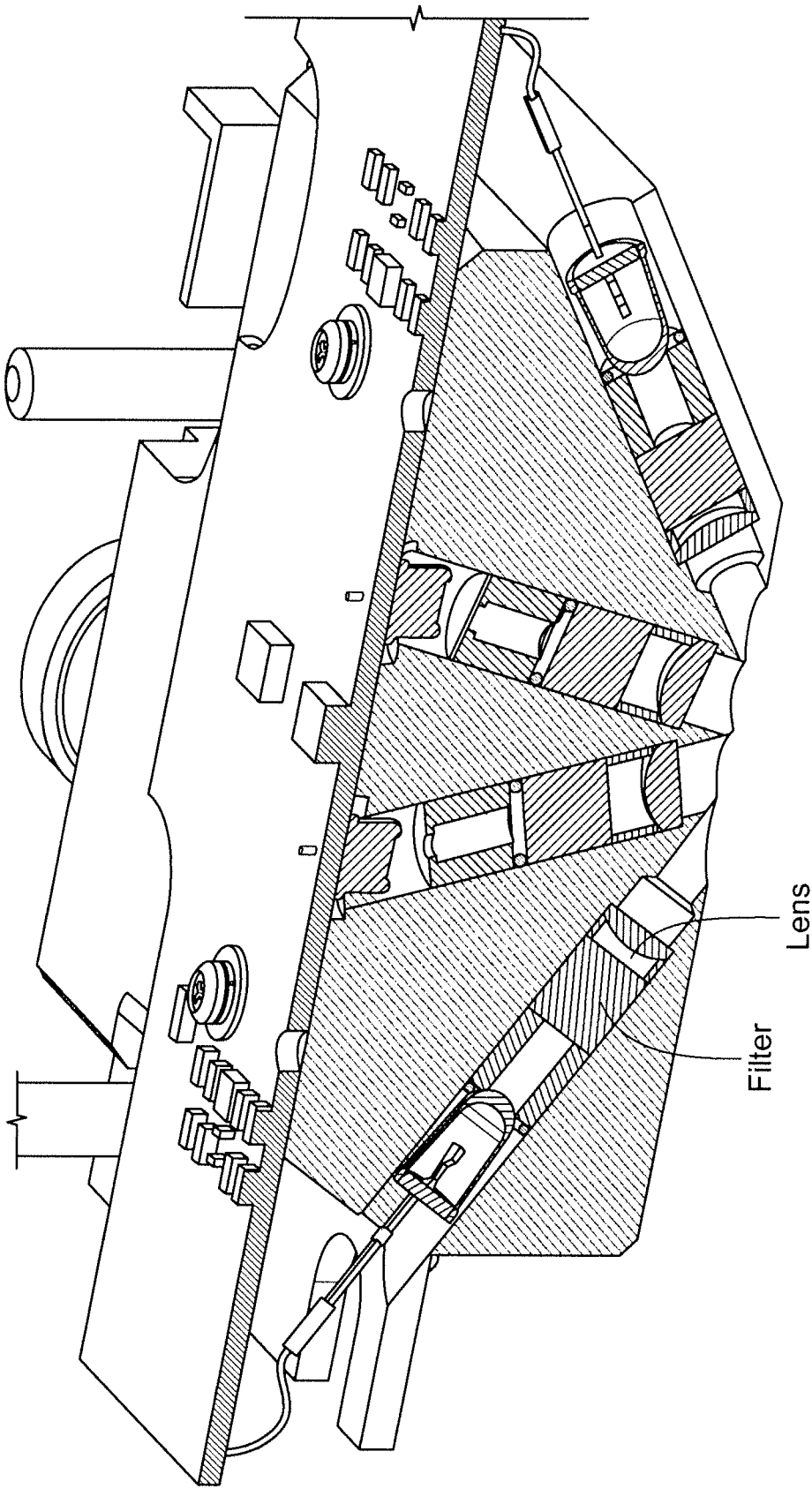


FIG. 59

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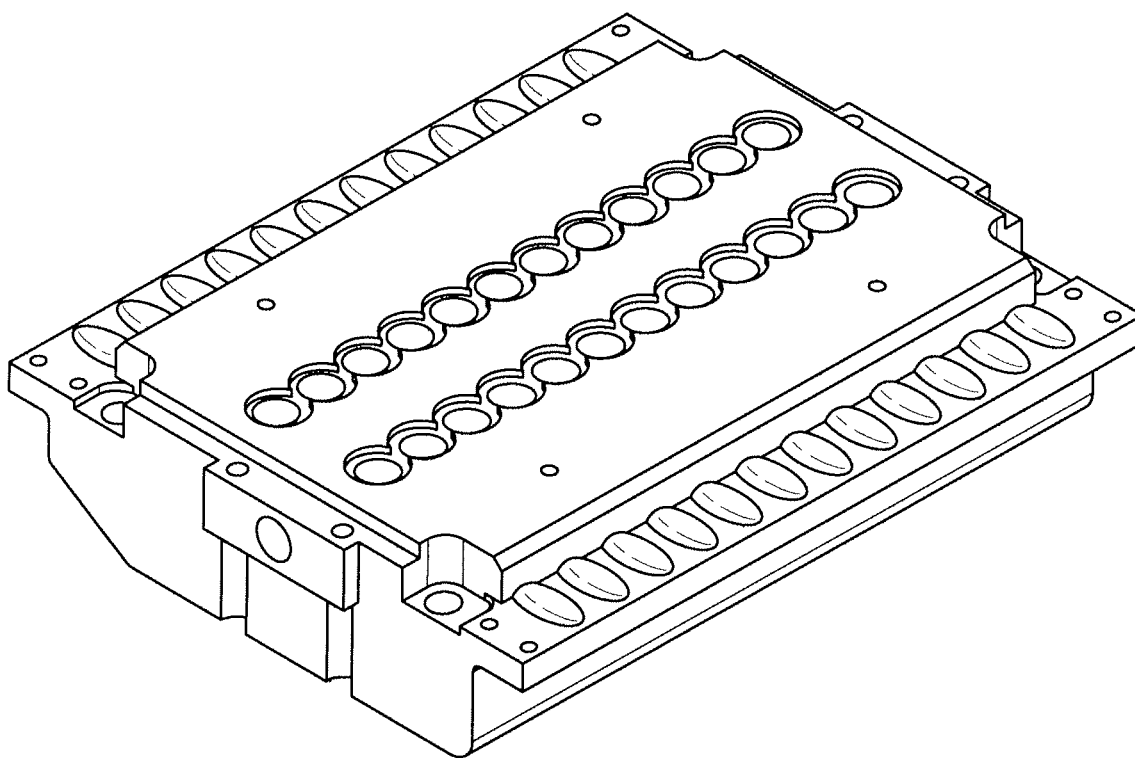


FIG. 60

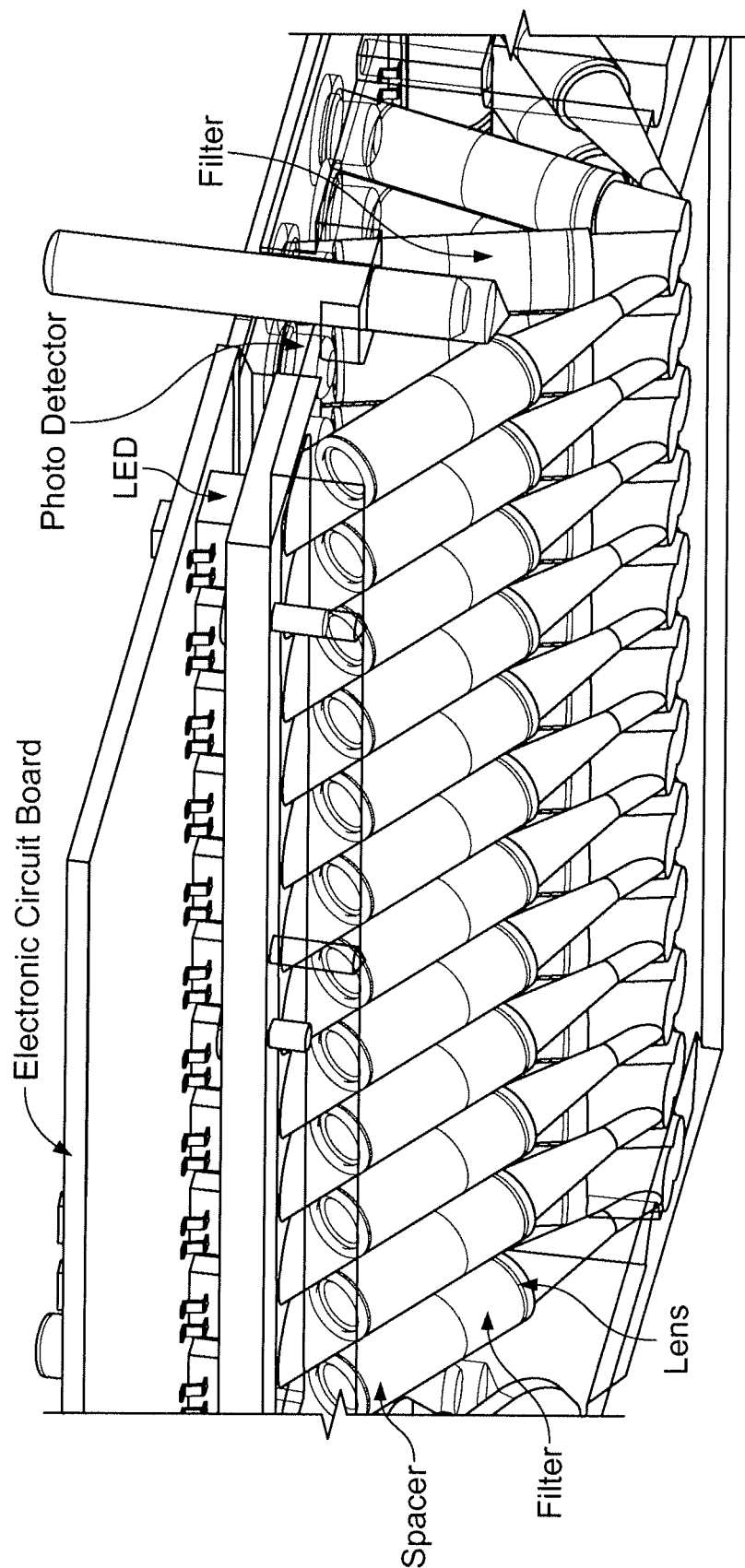


FIG. 61

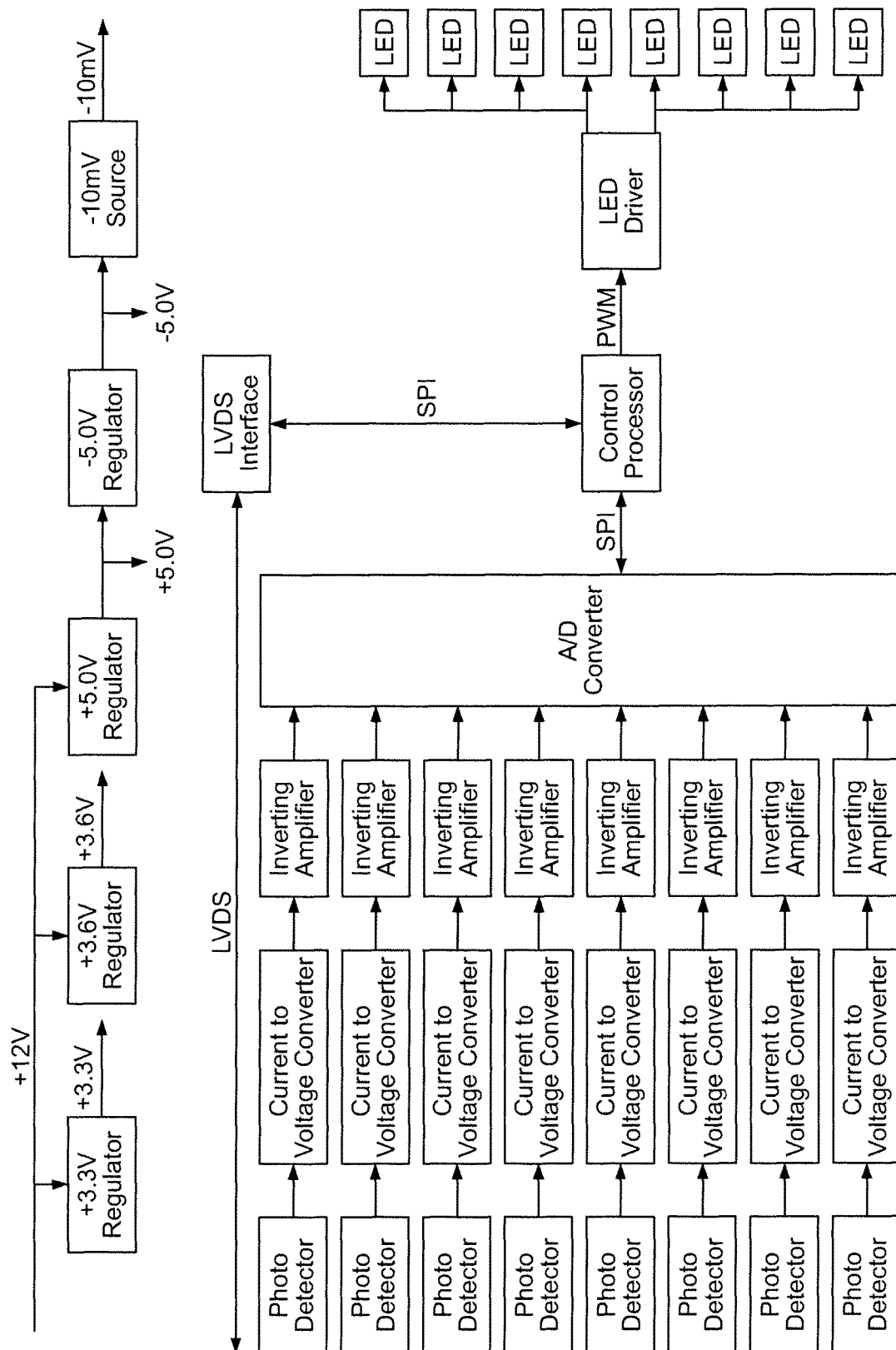


FIG. 62

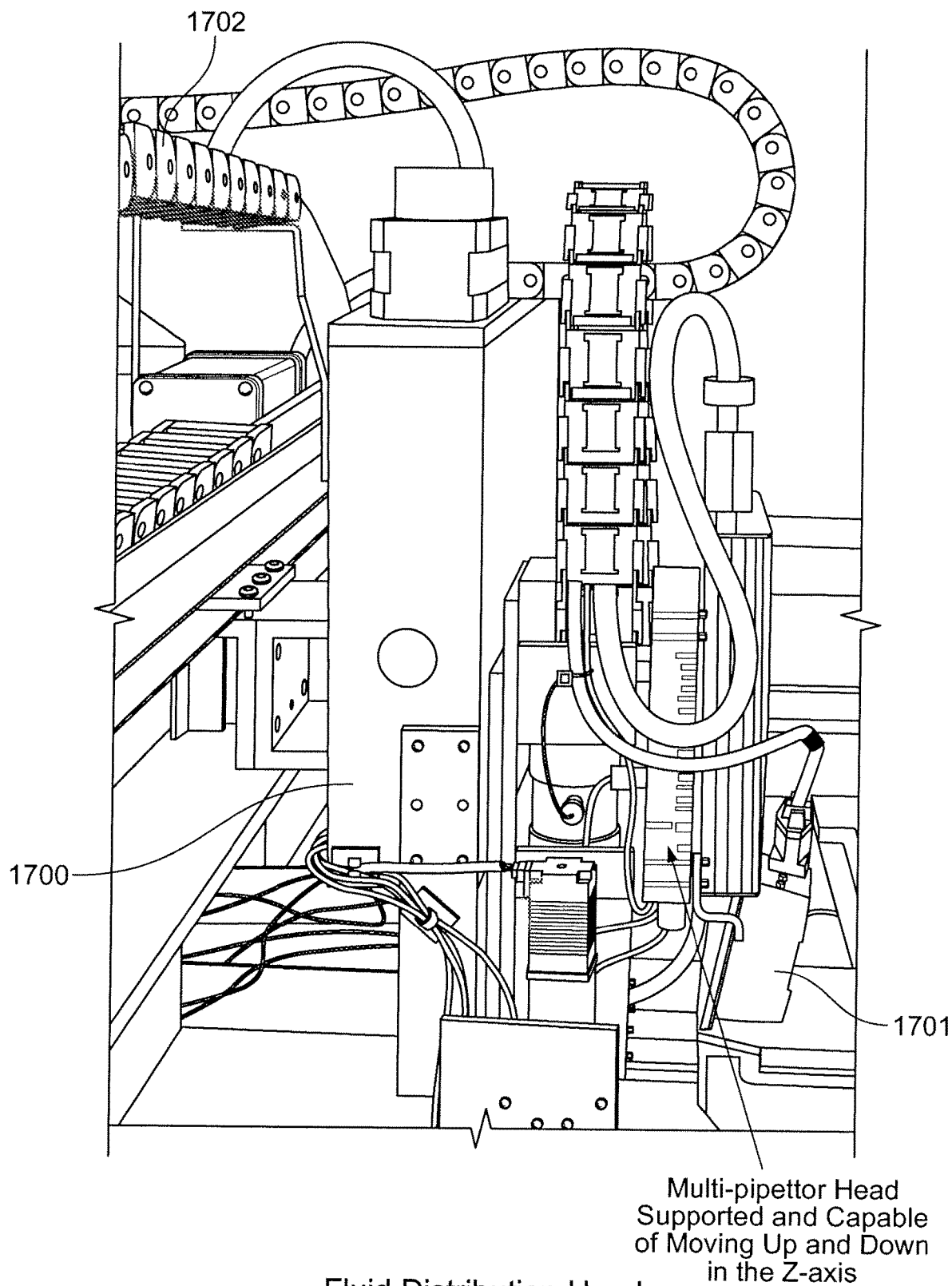


FIG. 63

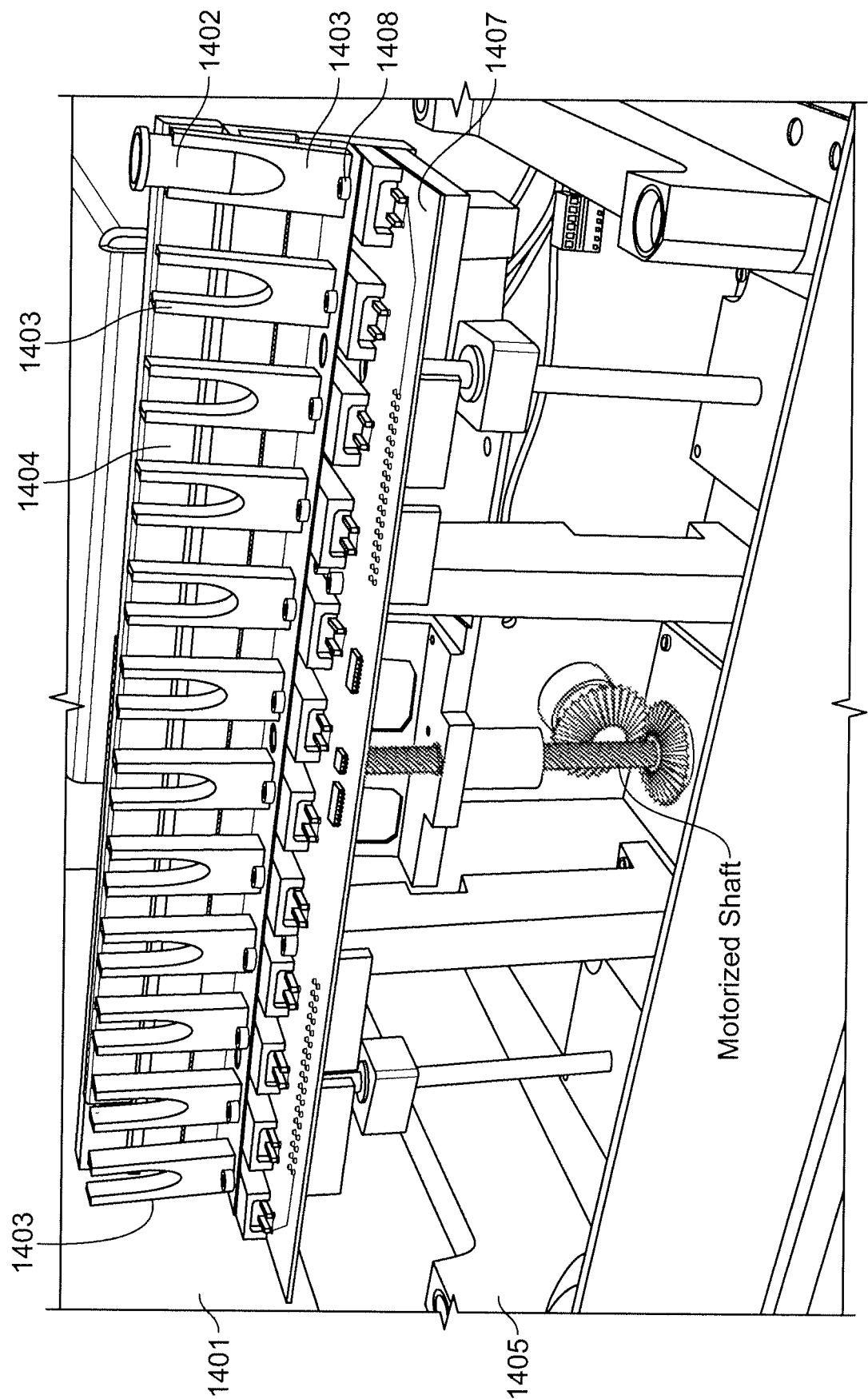


FIG. 64

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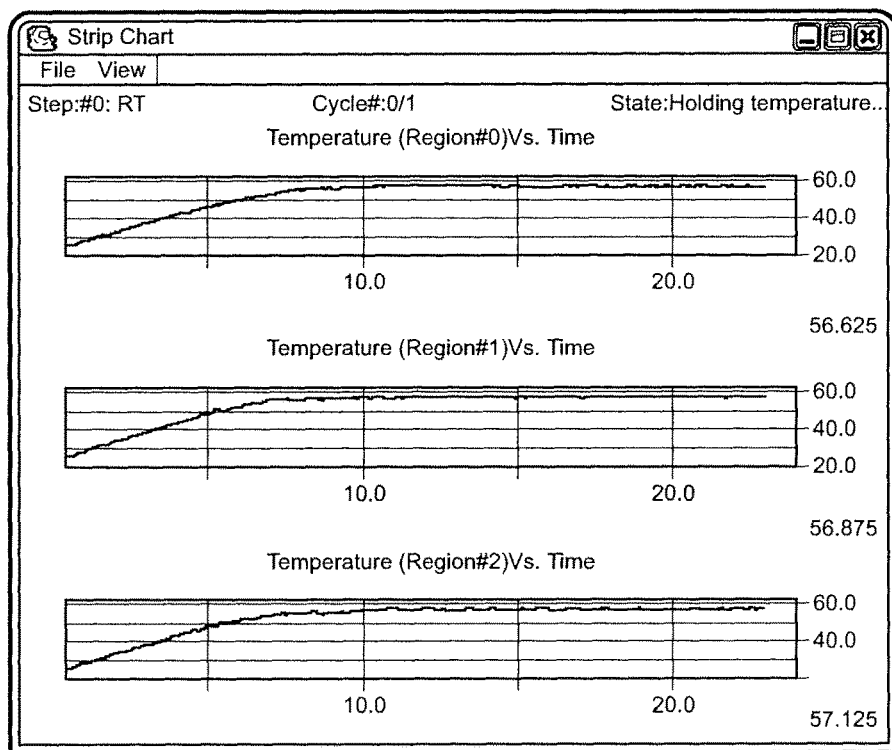


FIG. 65A

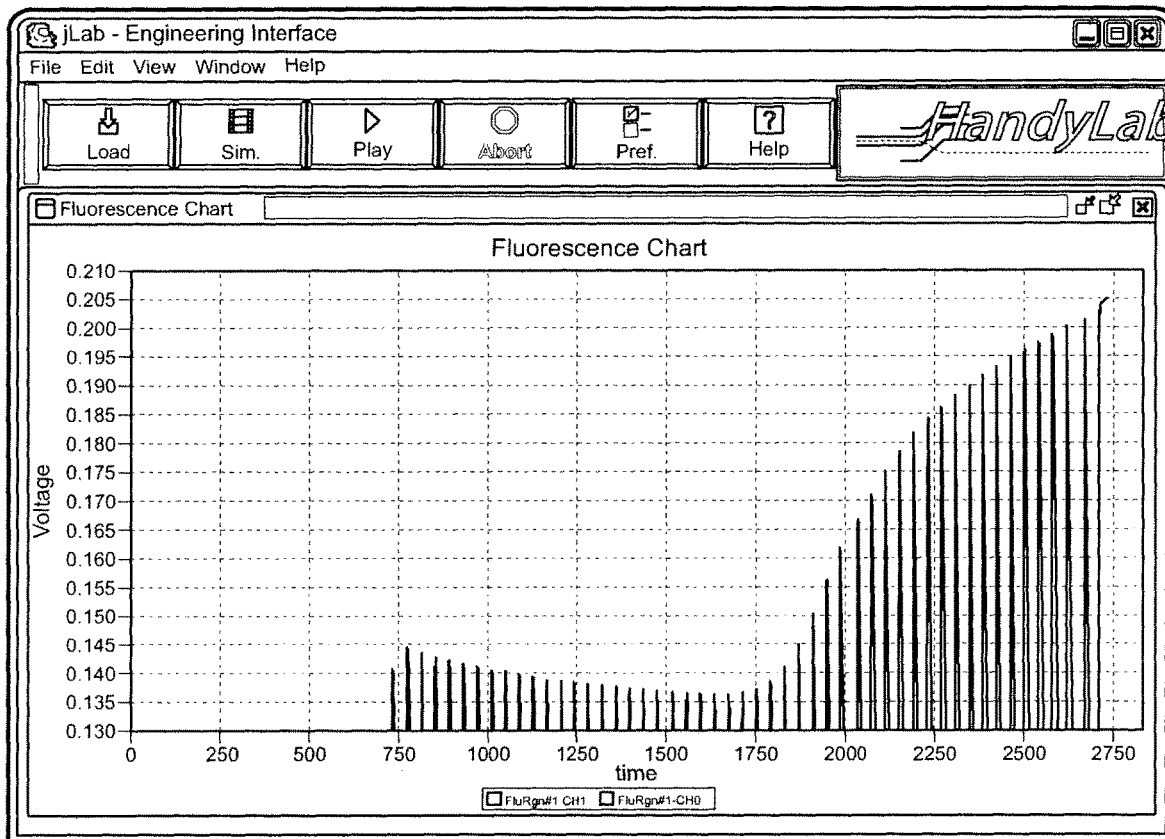


FIG. 65B

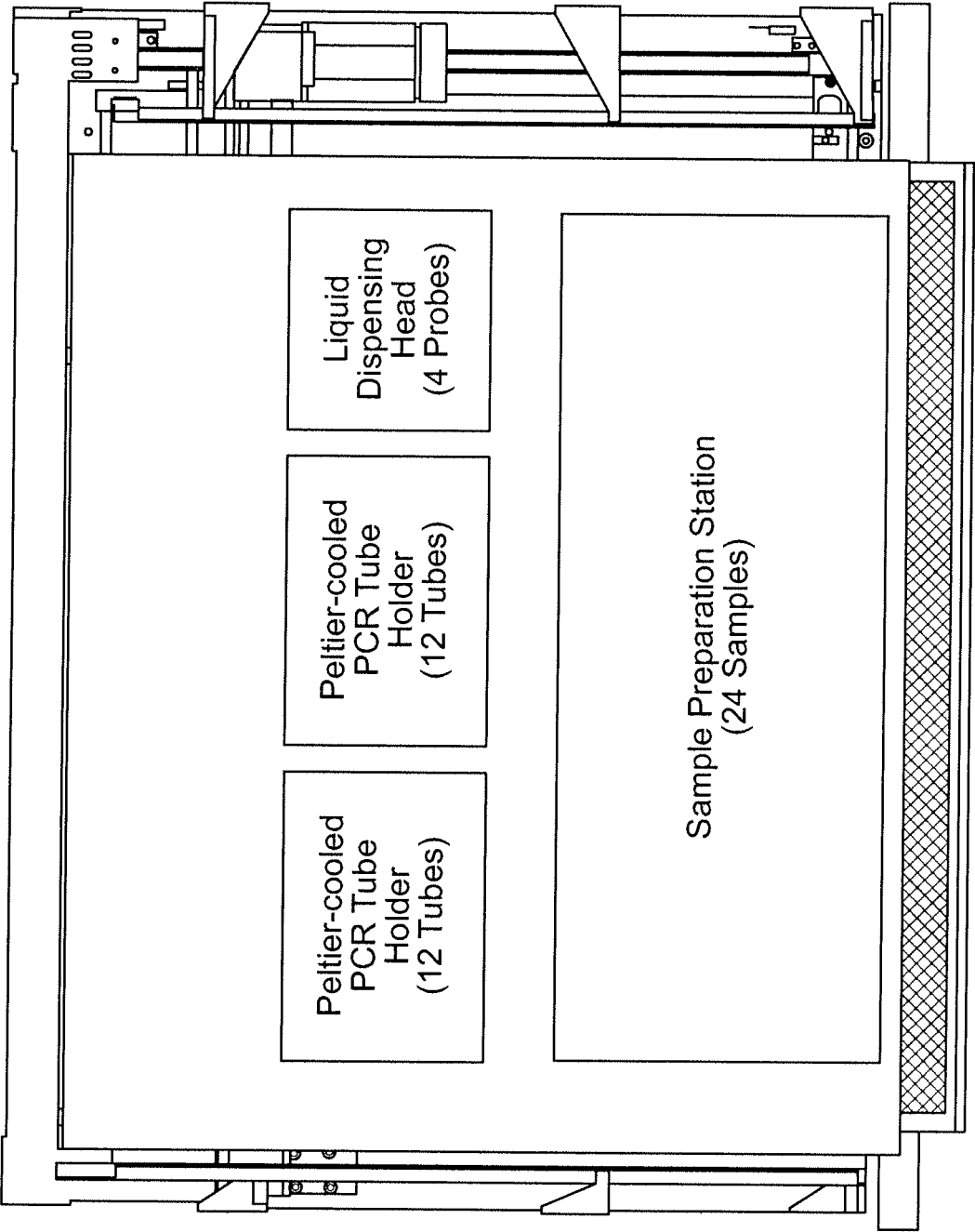
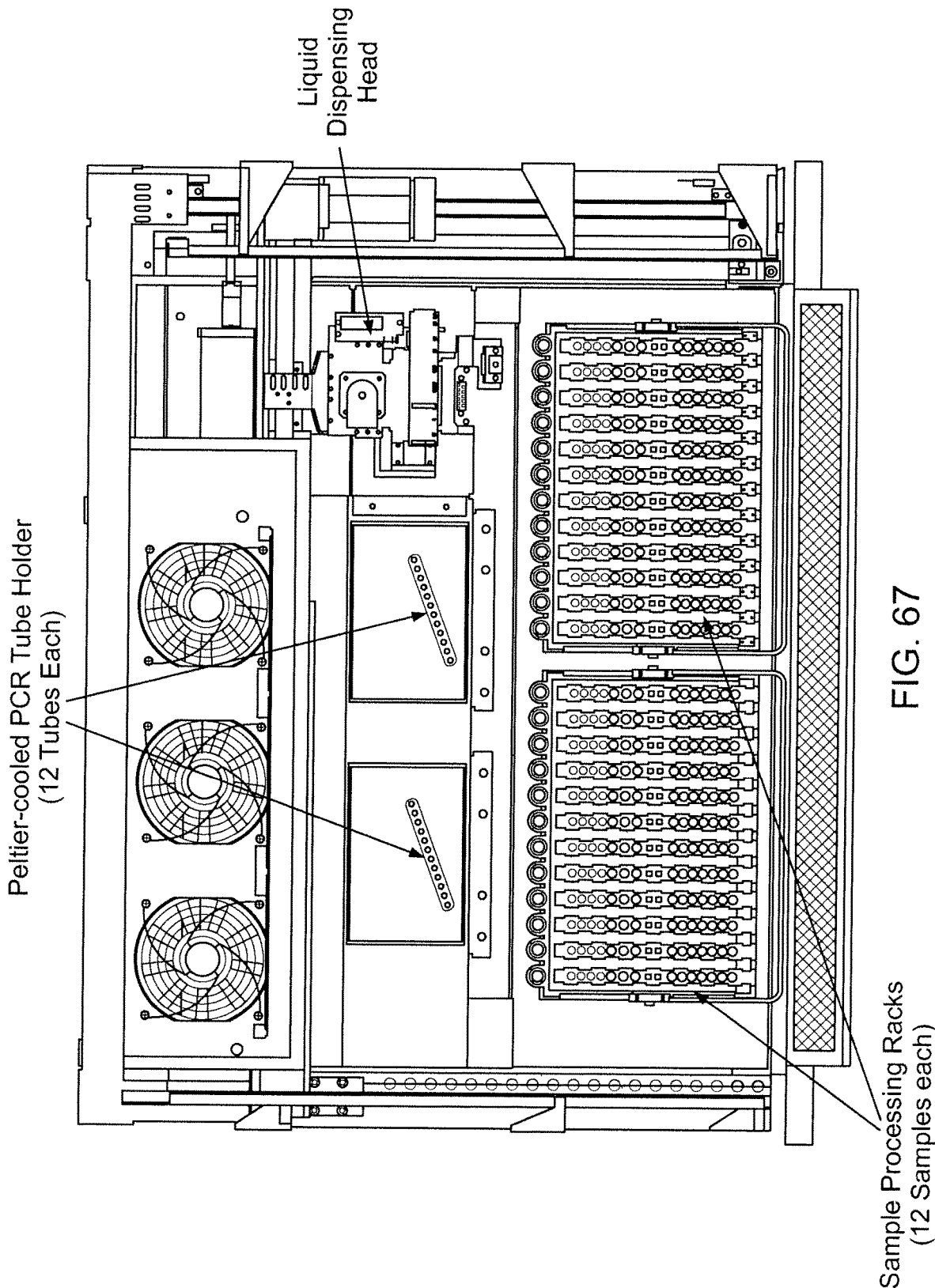


FIG. 66



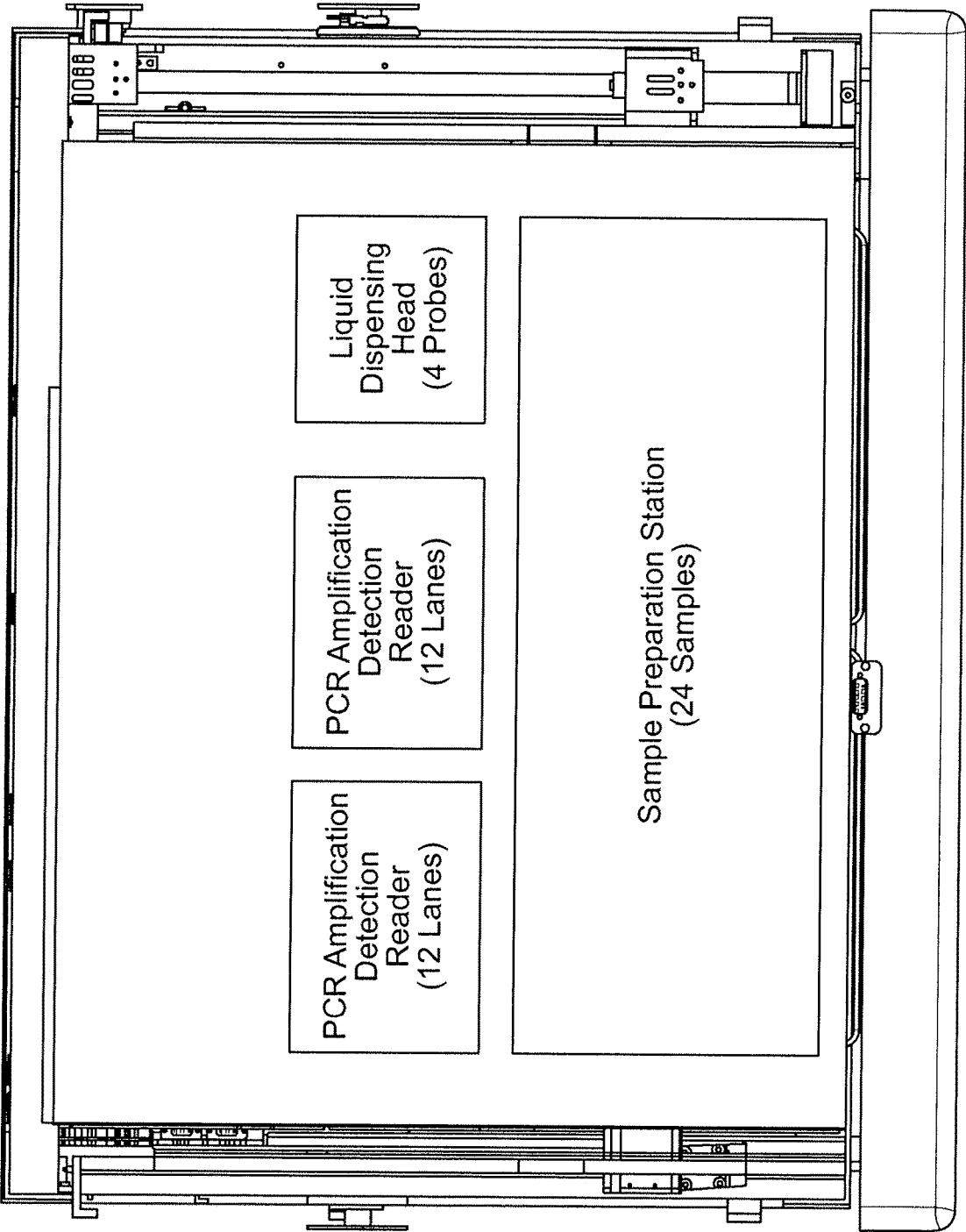


FIG. 68

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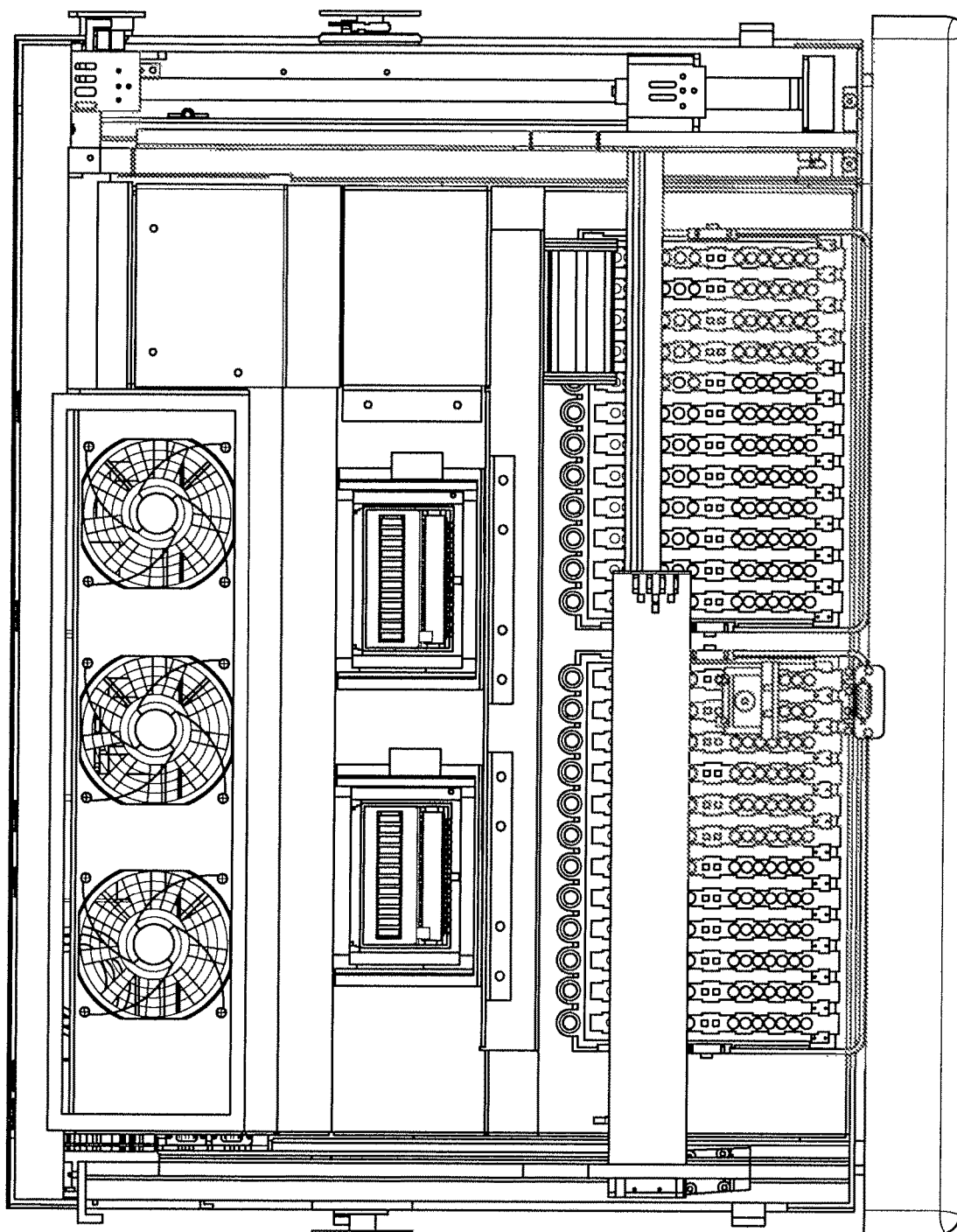


FIG. 69

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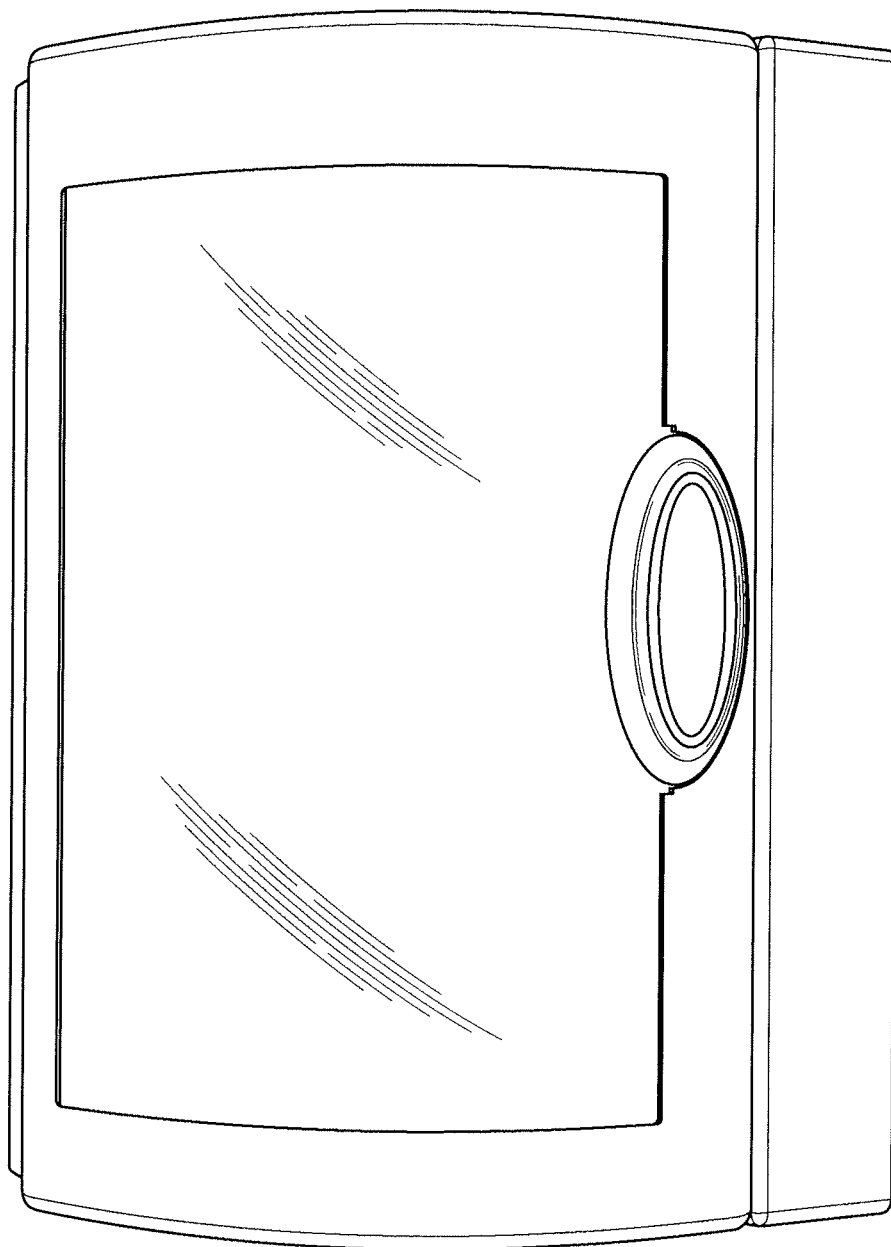


FIG. 70

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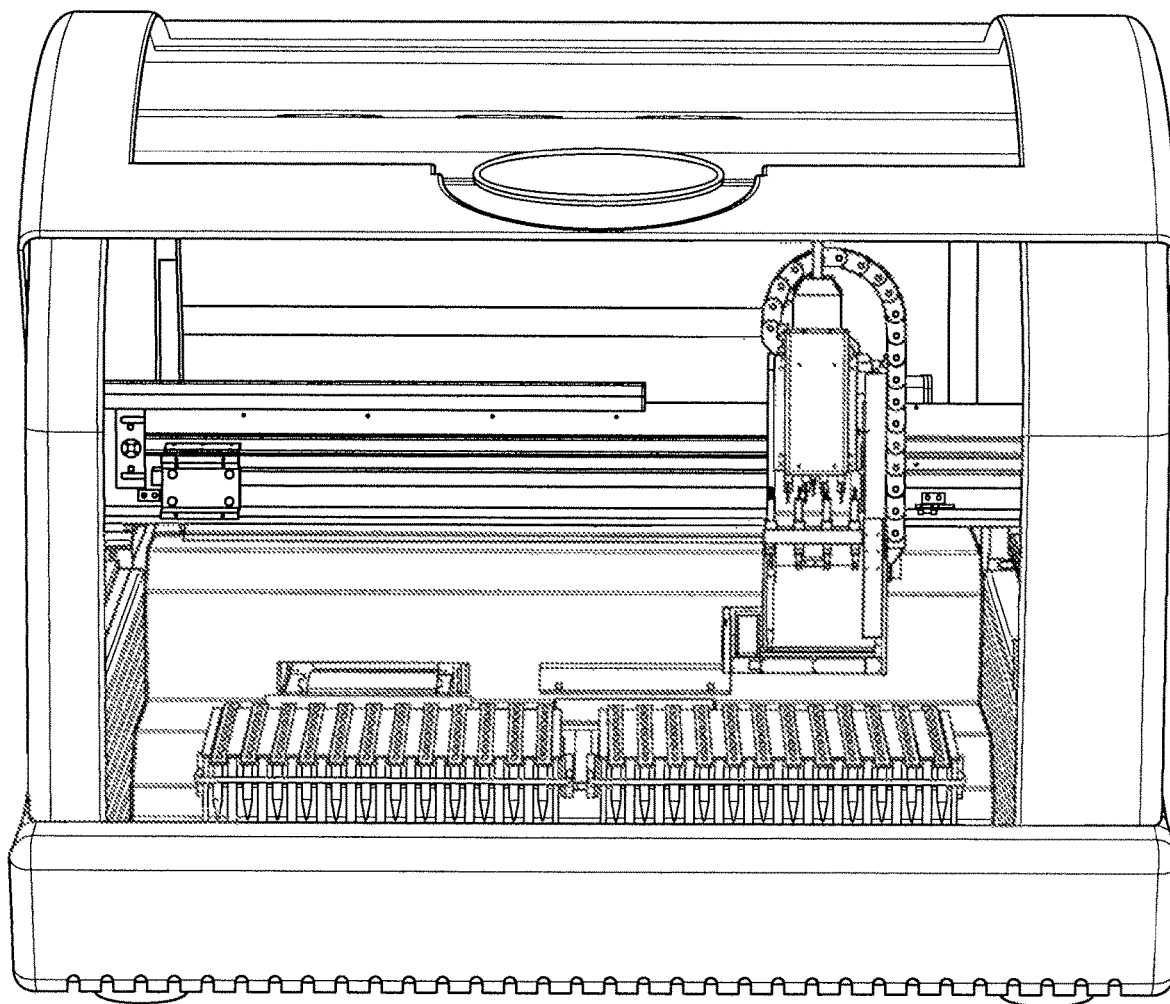
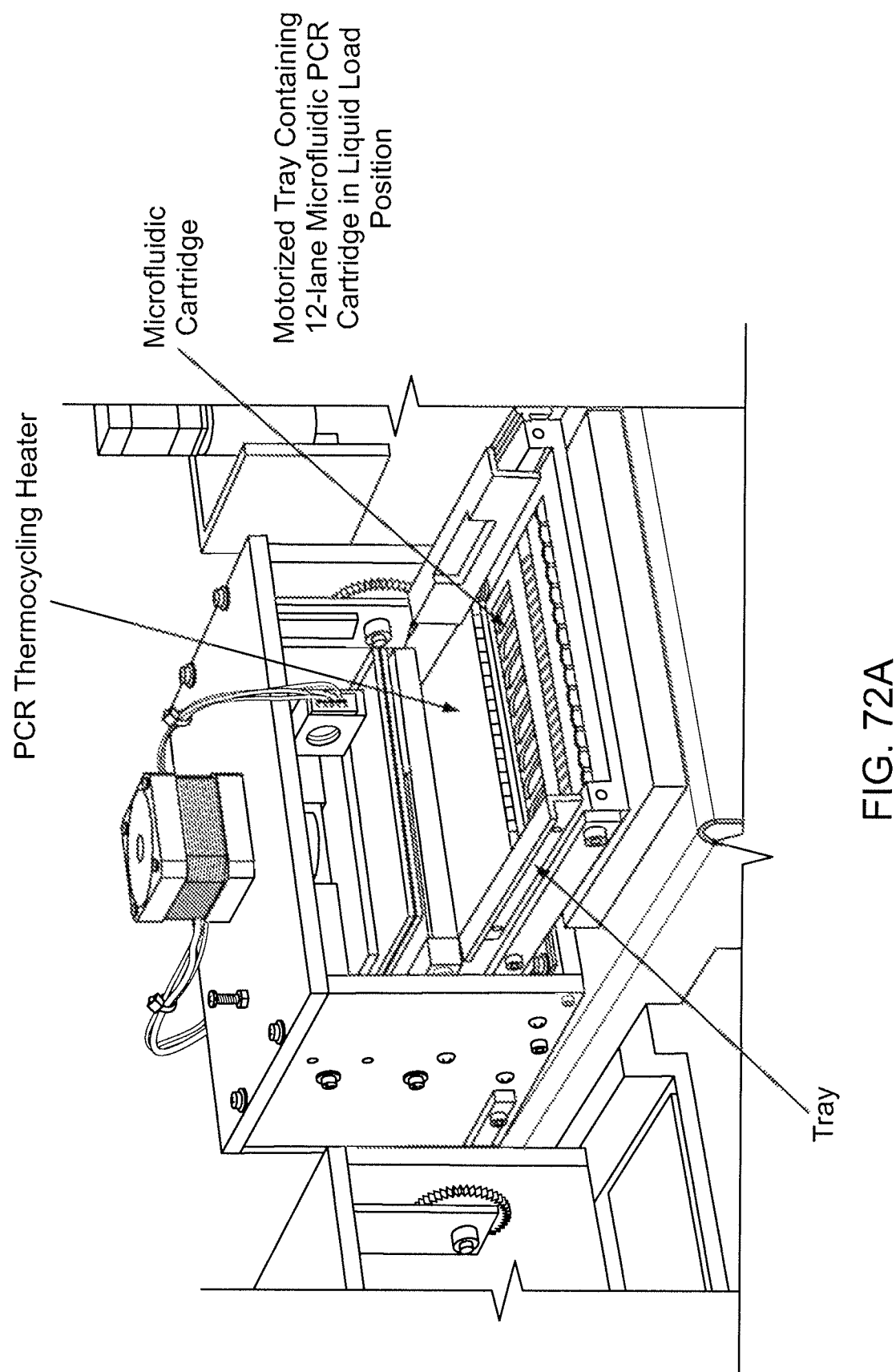
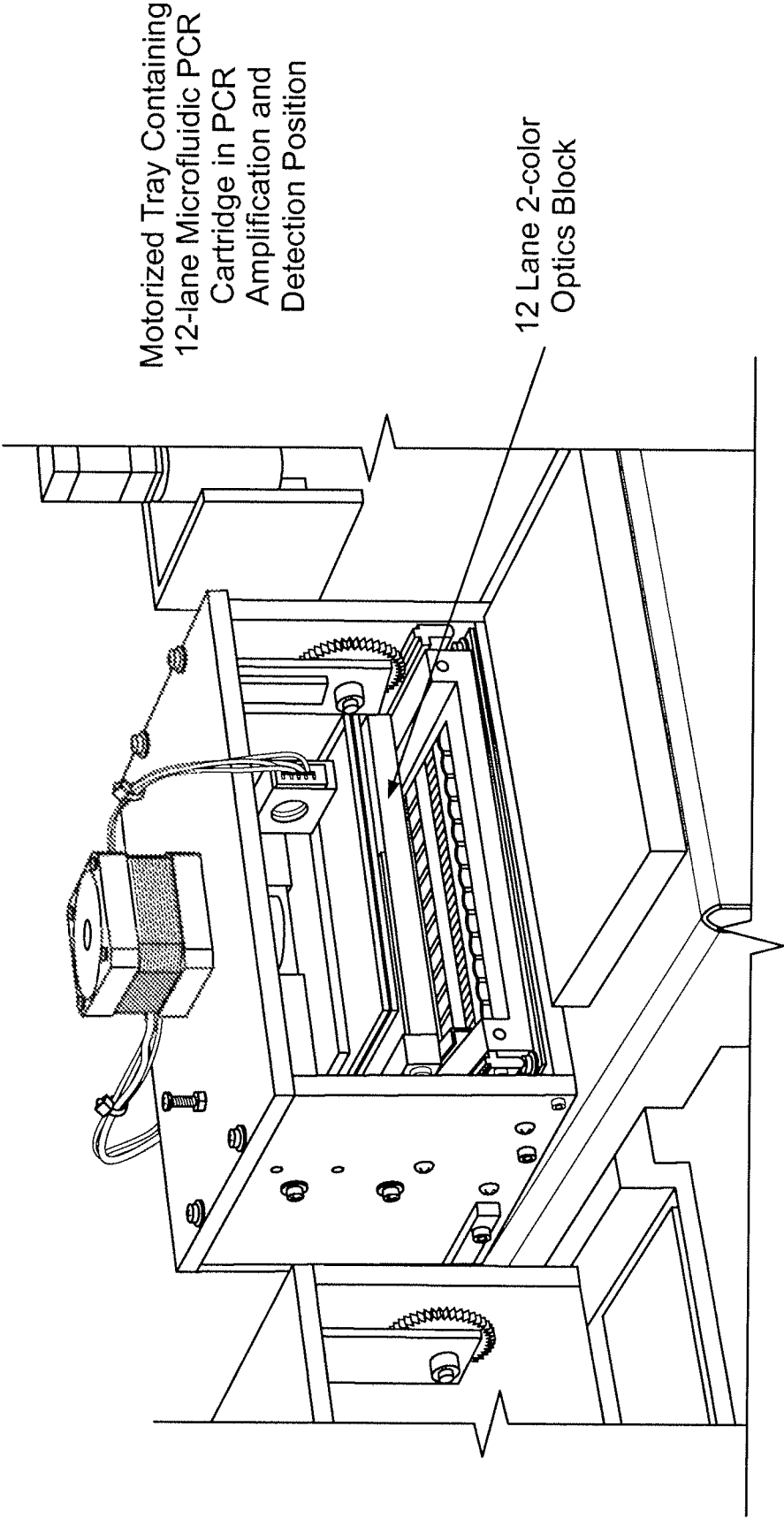


FIG. 71





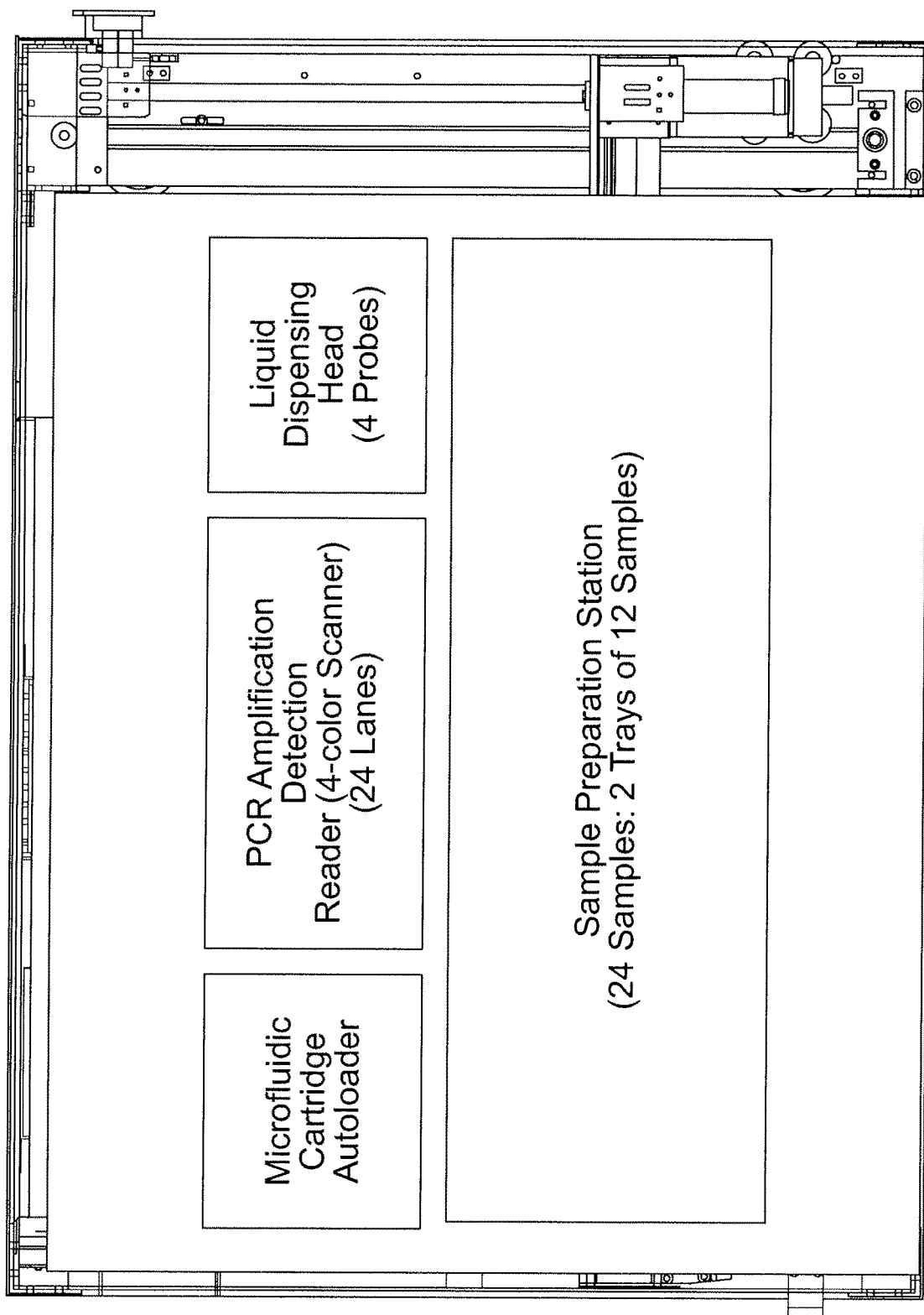


FIG. 73

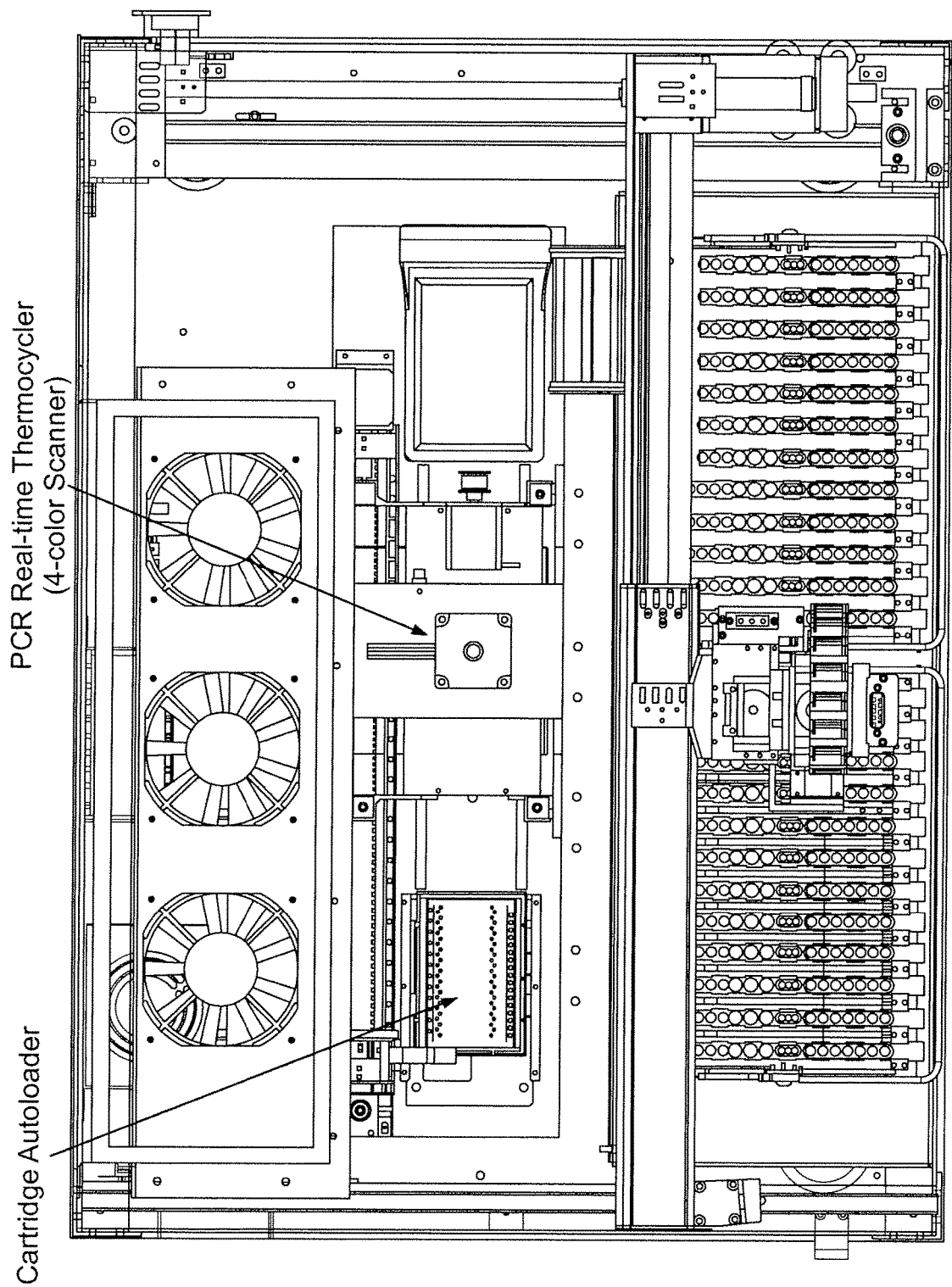


FIG. 74

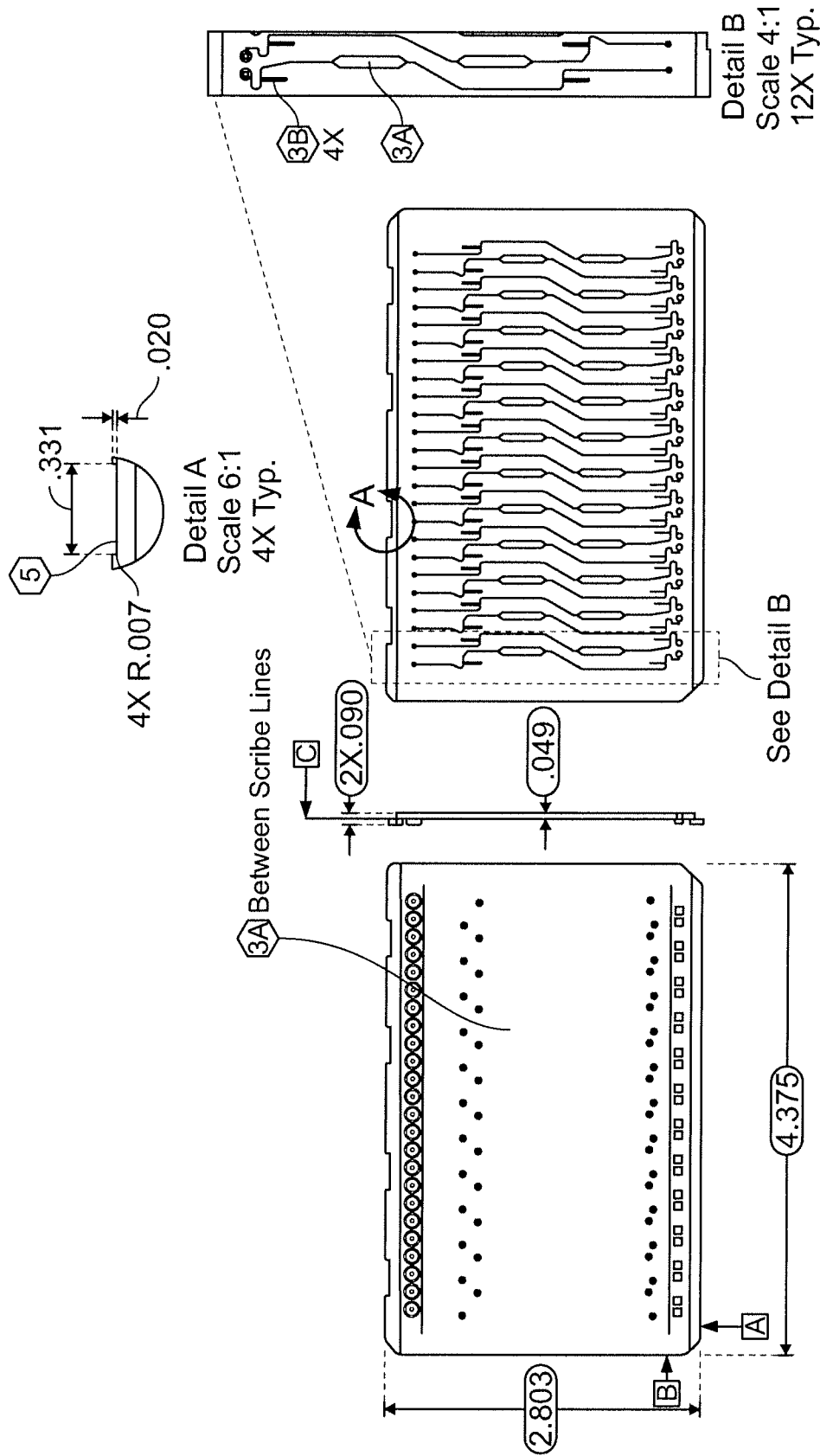


FIG. 75

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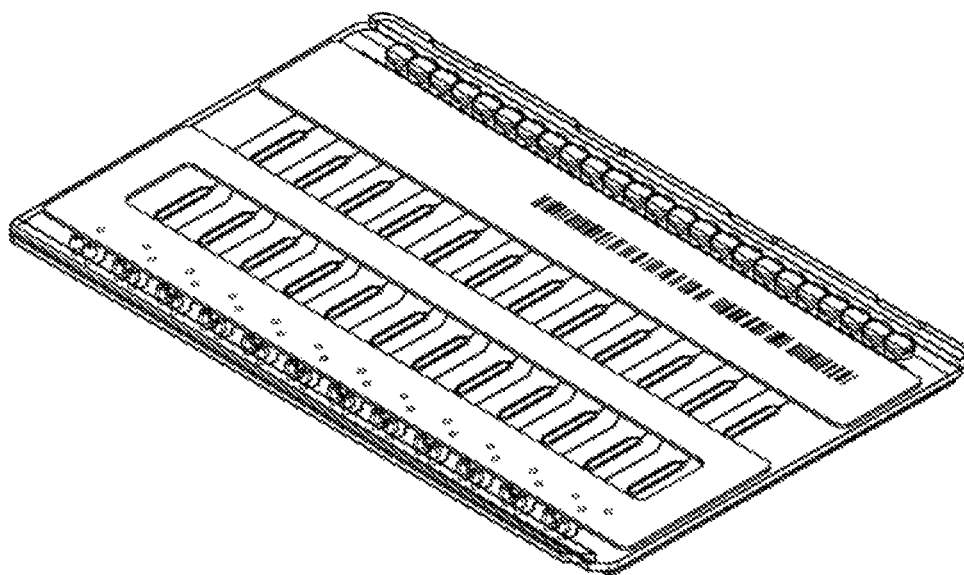


FIG. 76

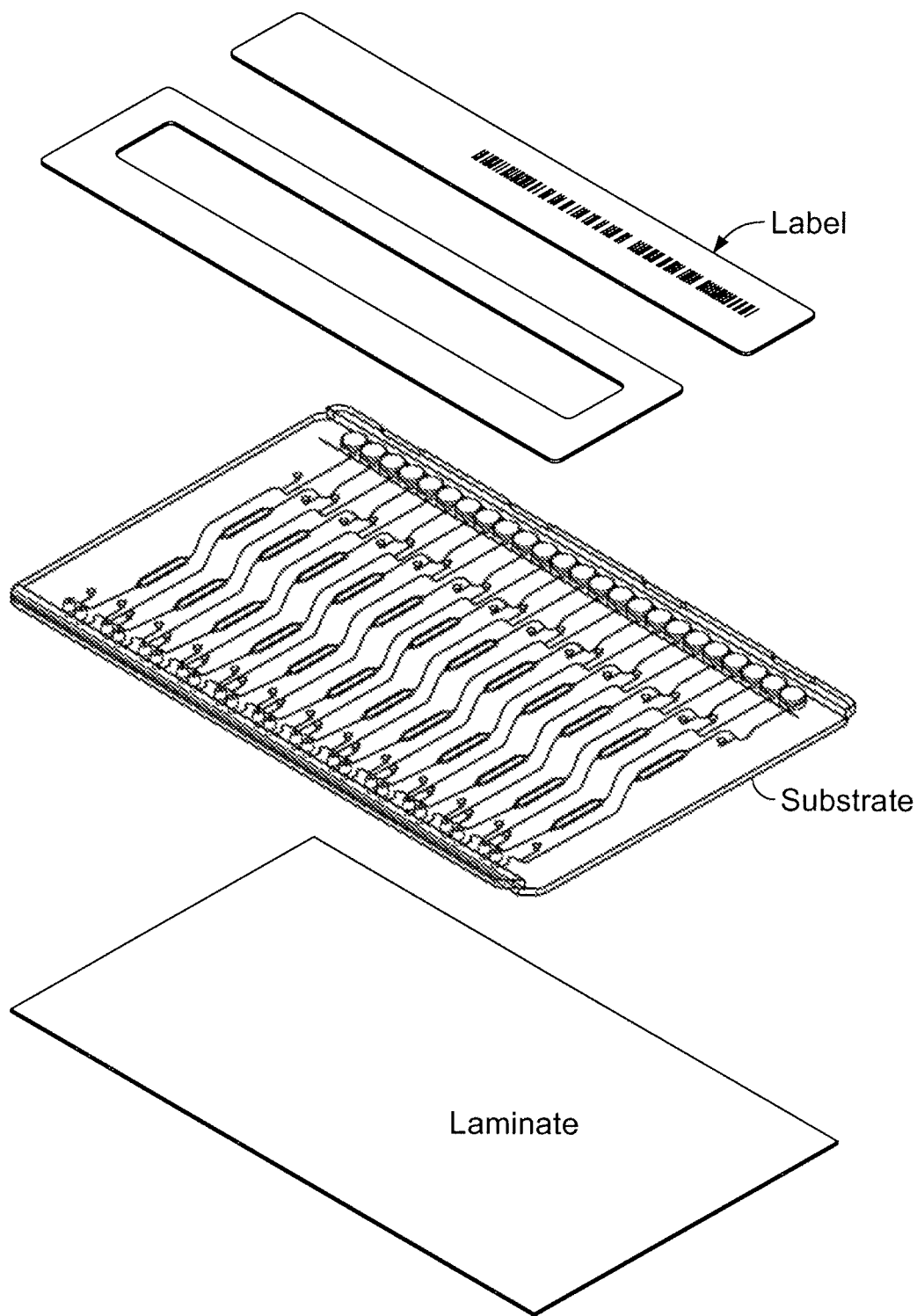
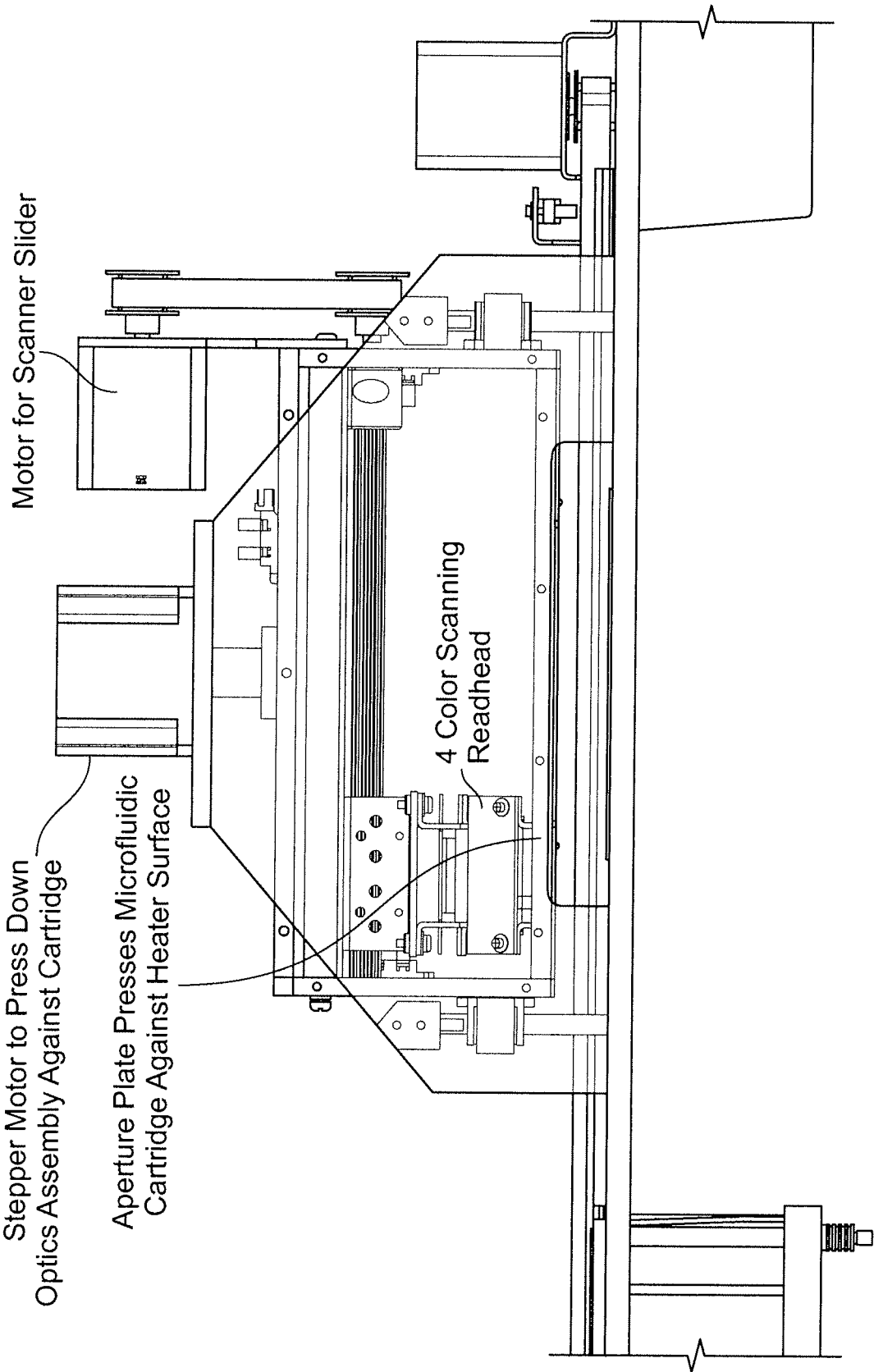


FIG. 77



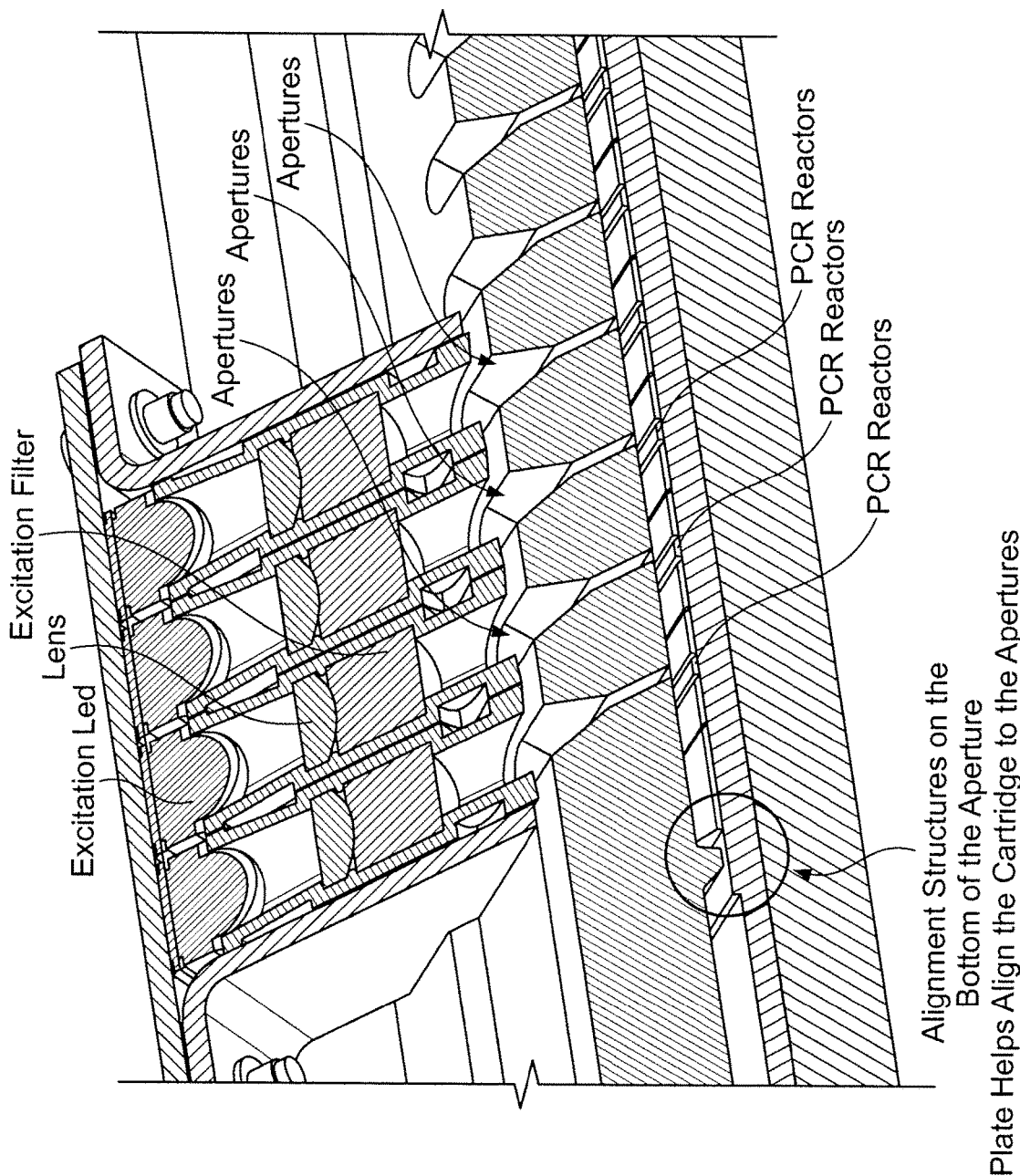


FIG. 79A

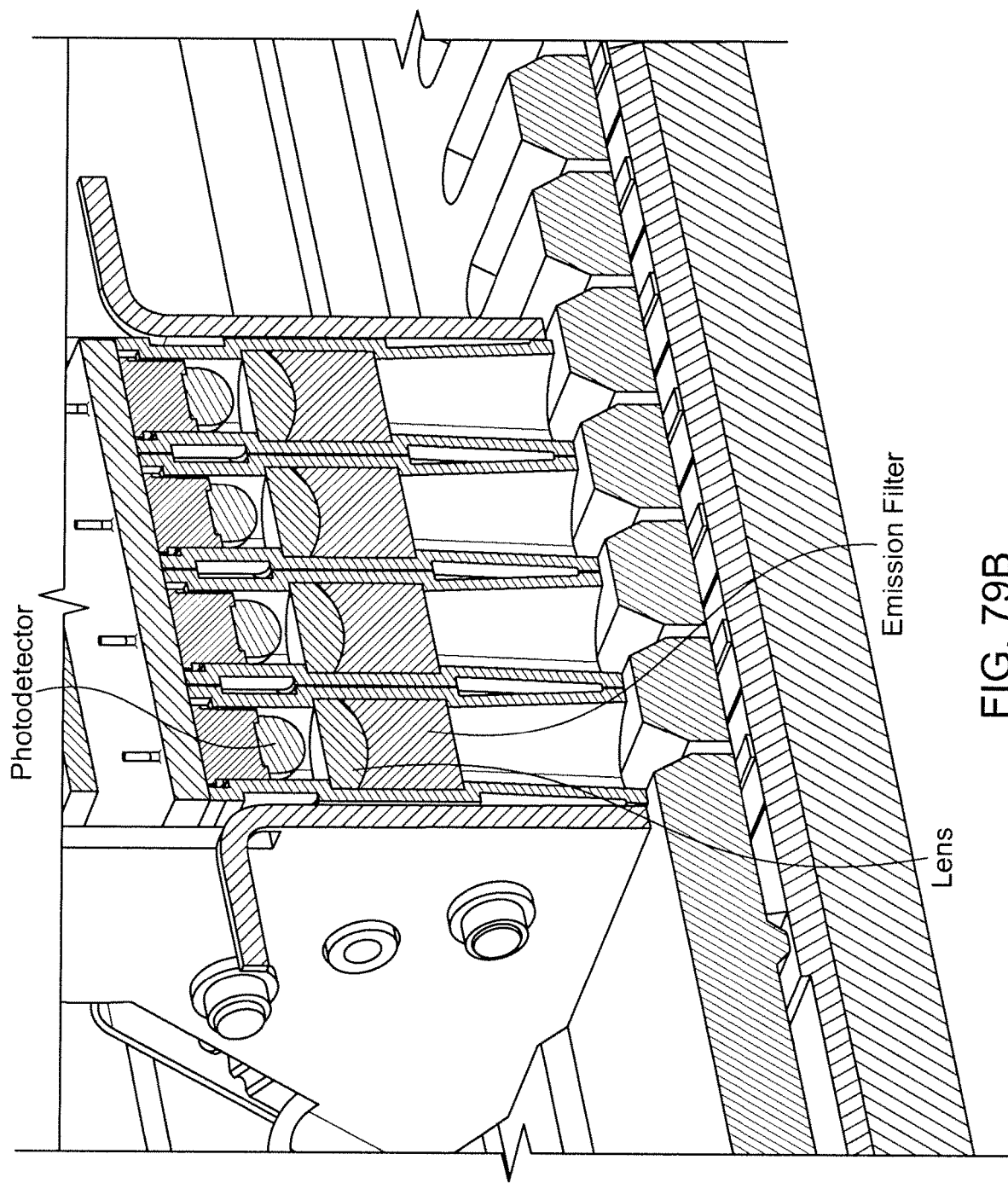
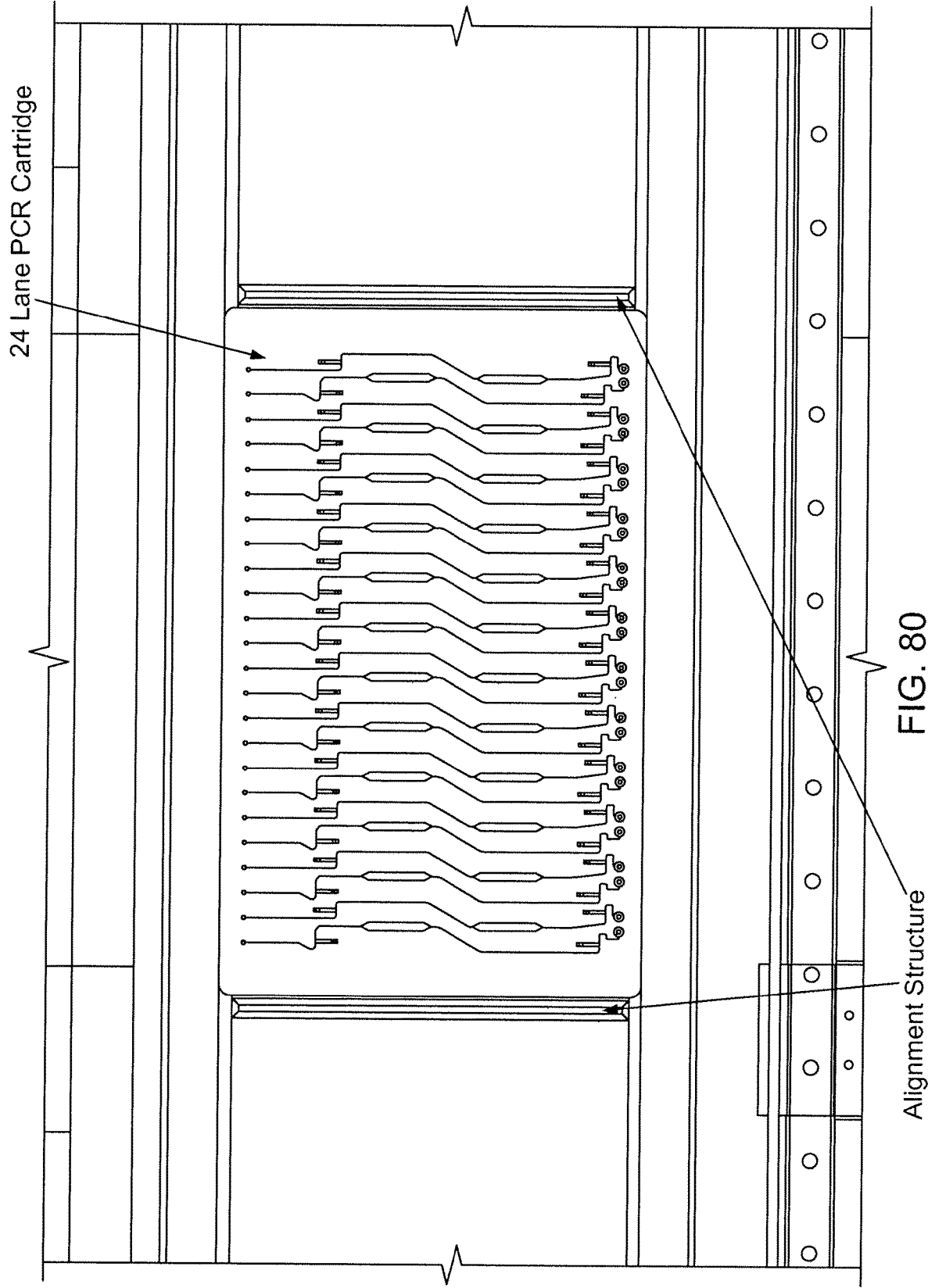


FIG. 79B



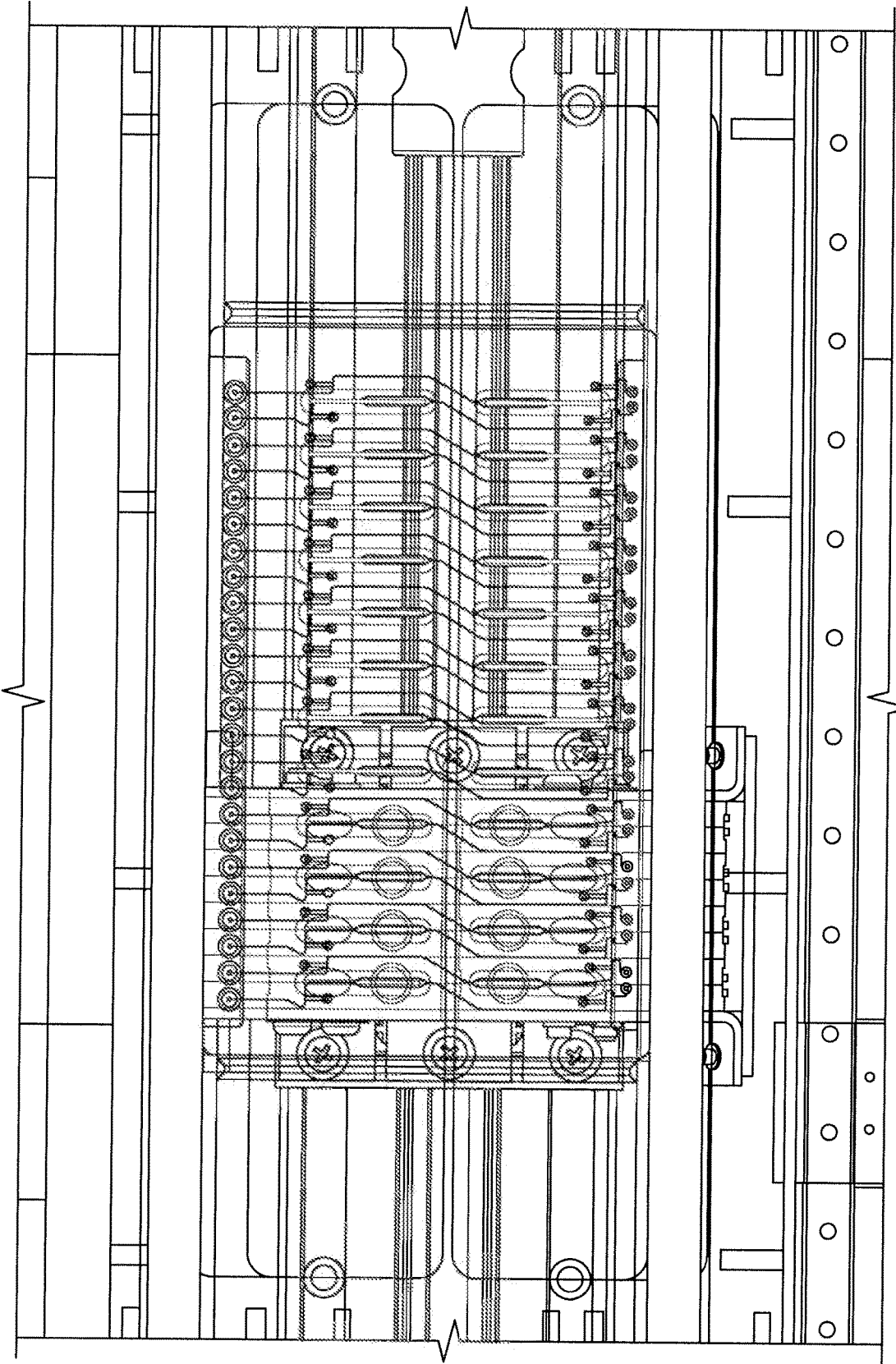


FIG. 81

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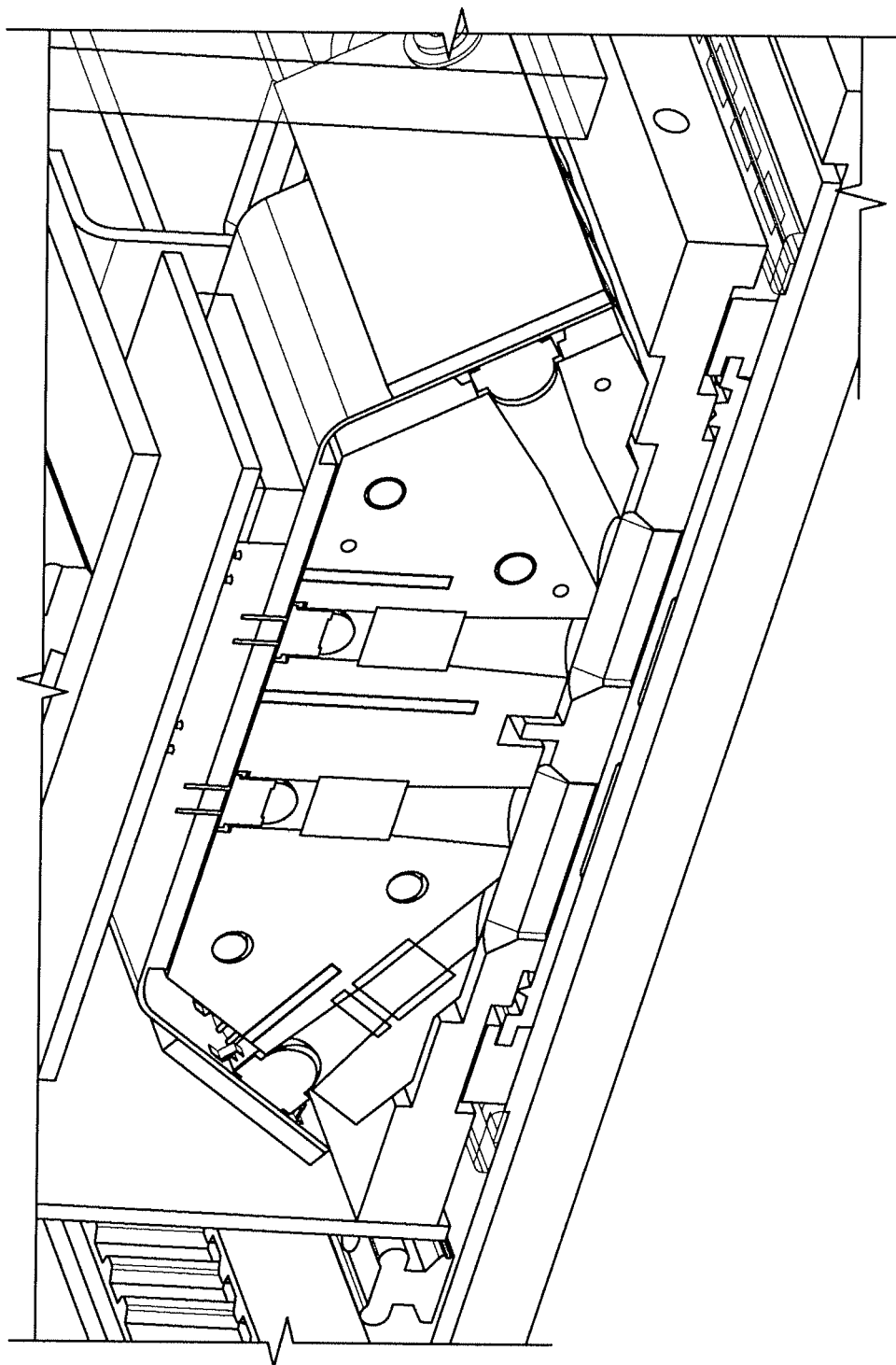


FIG. 82

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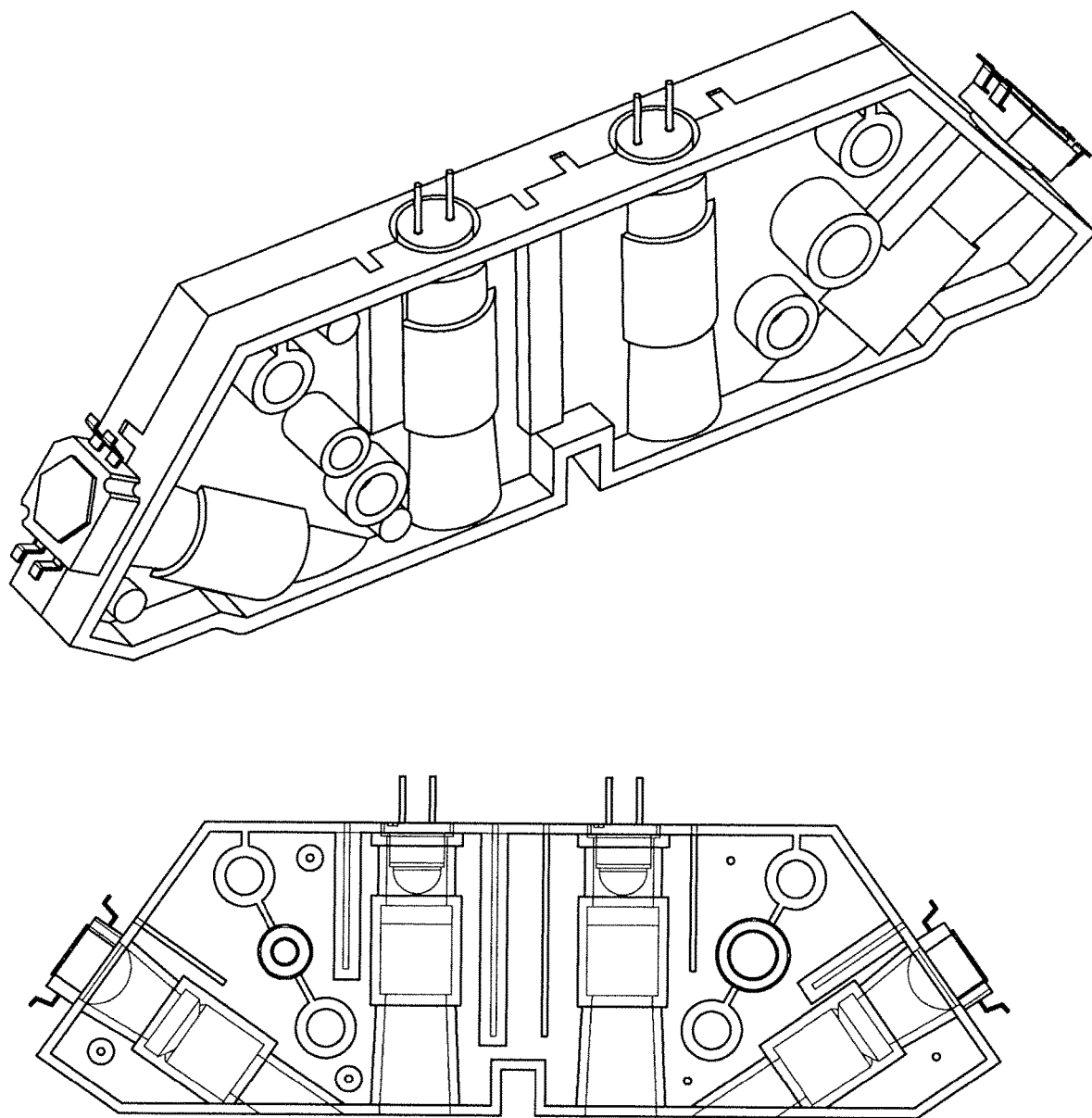


FIG. 83

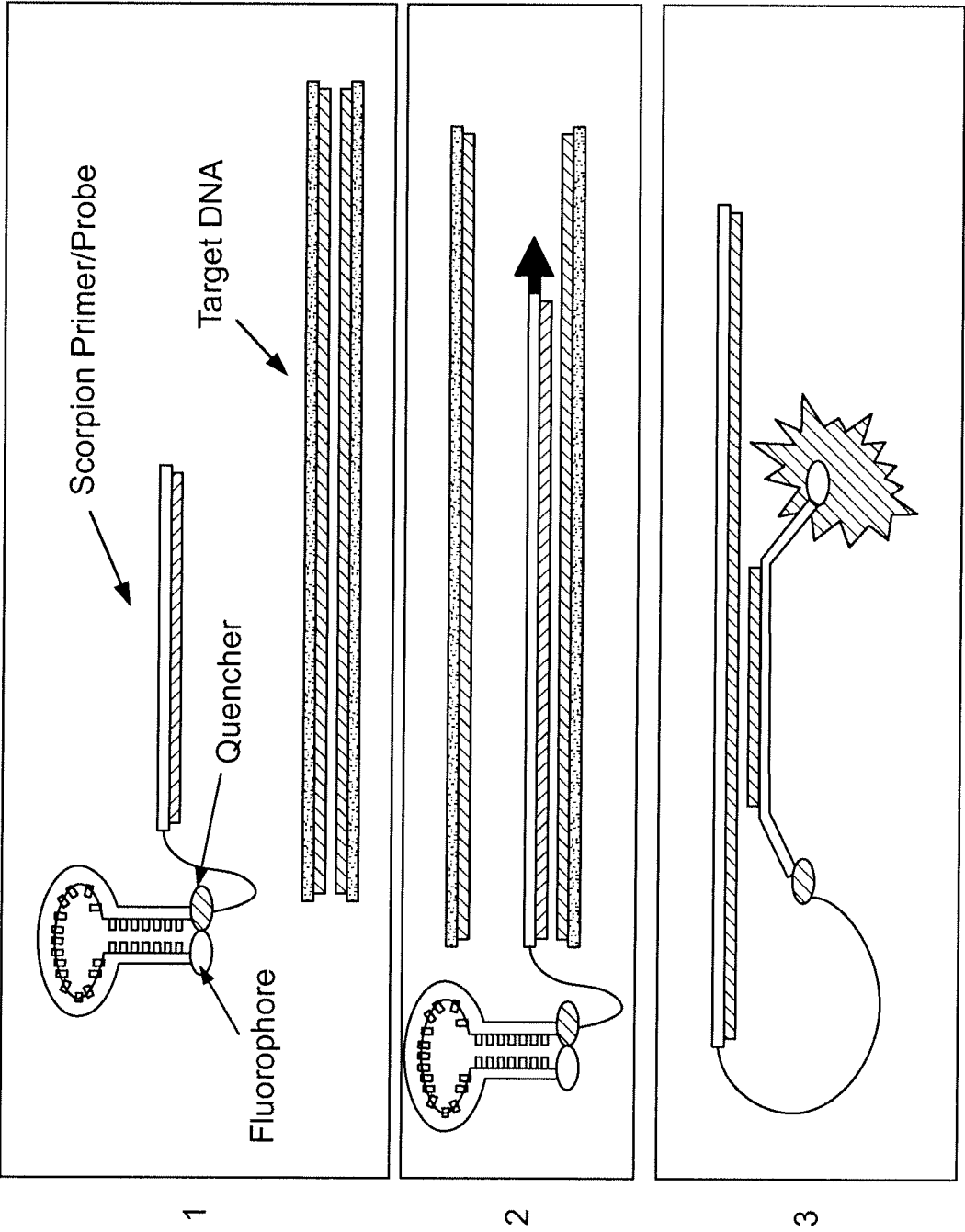


FIG. 84

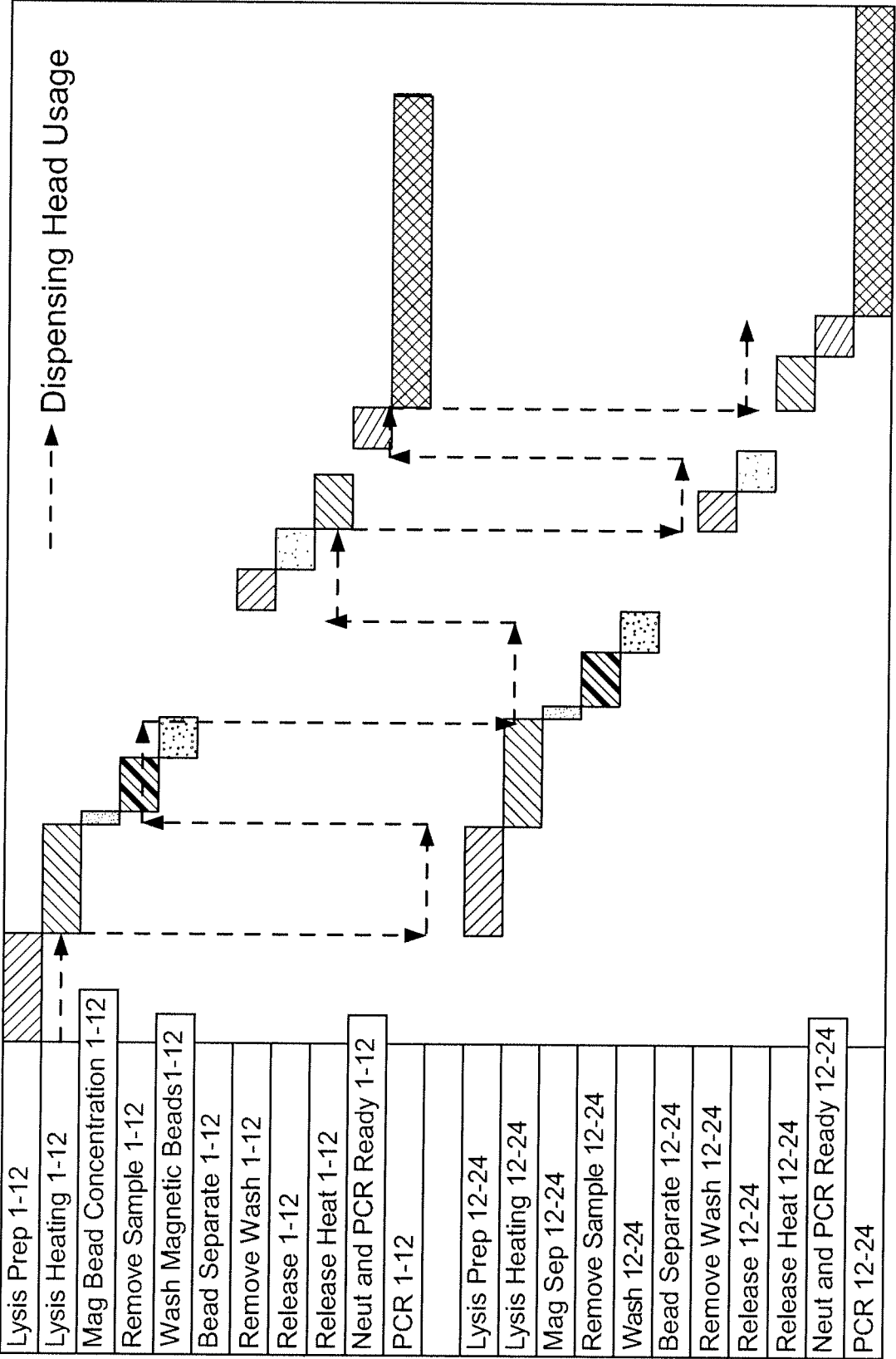


FIG. 85A

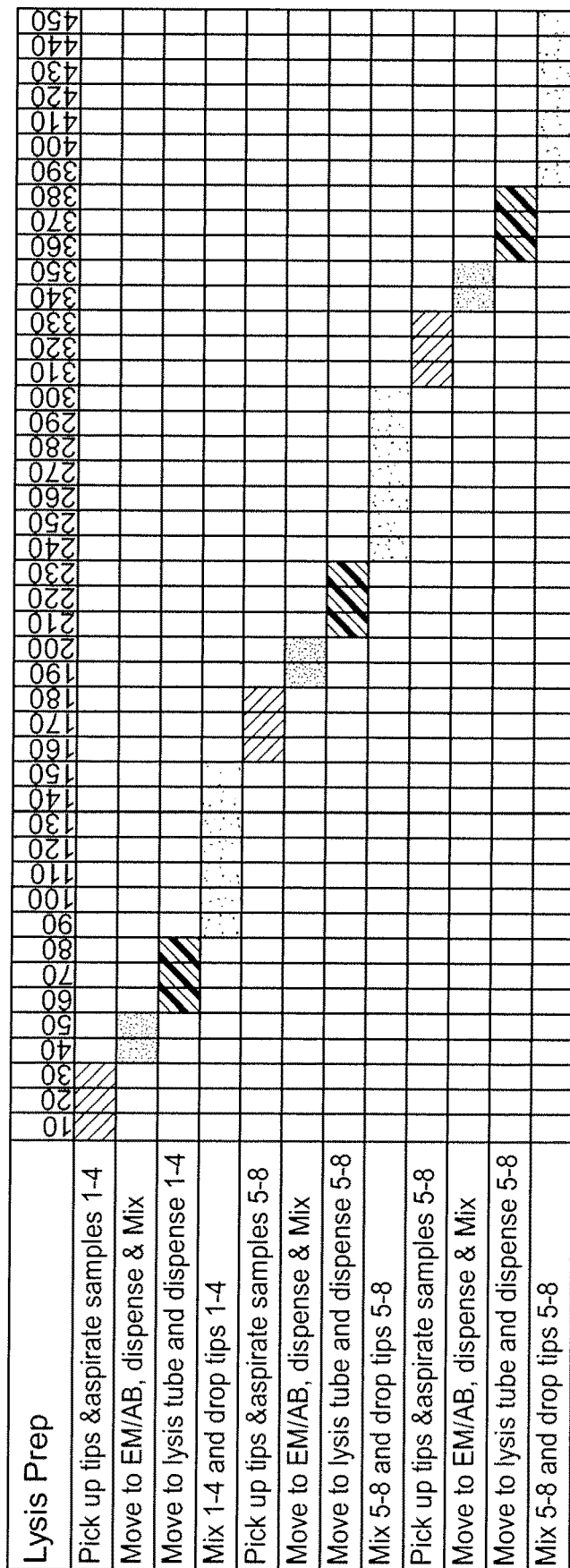


FIG. 85B

[illegible]

FIG. 85C

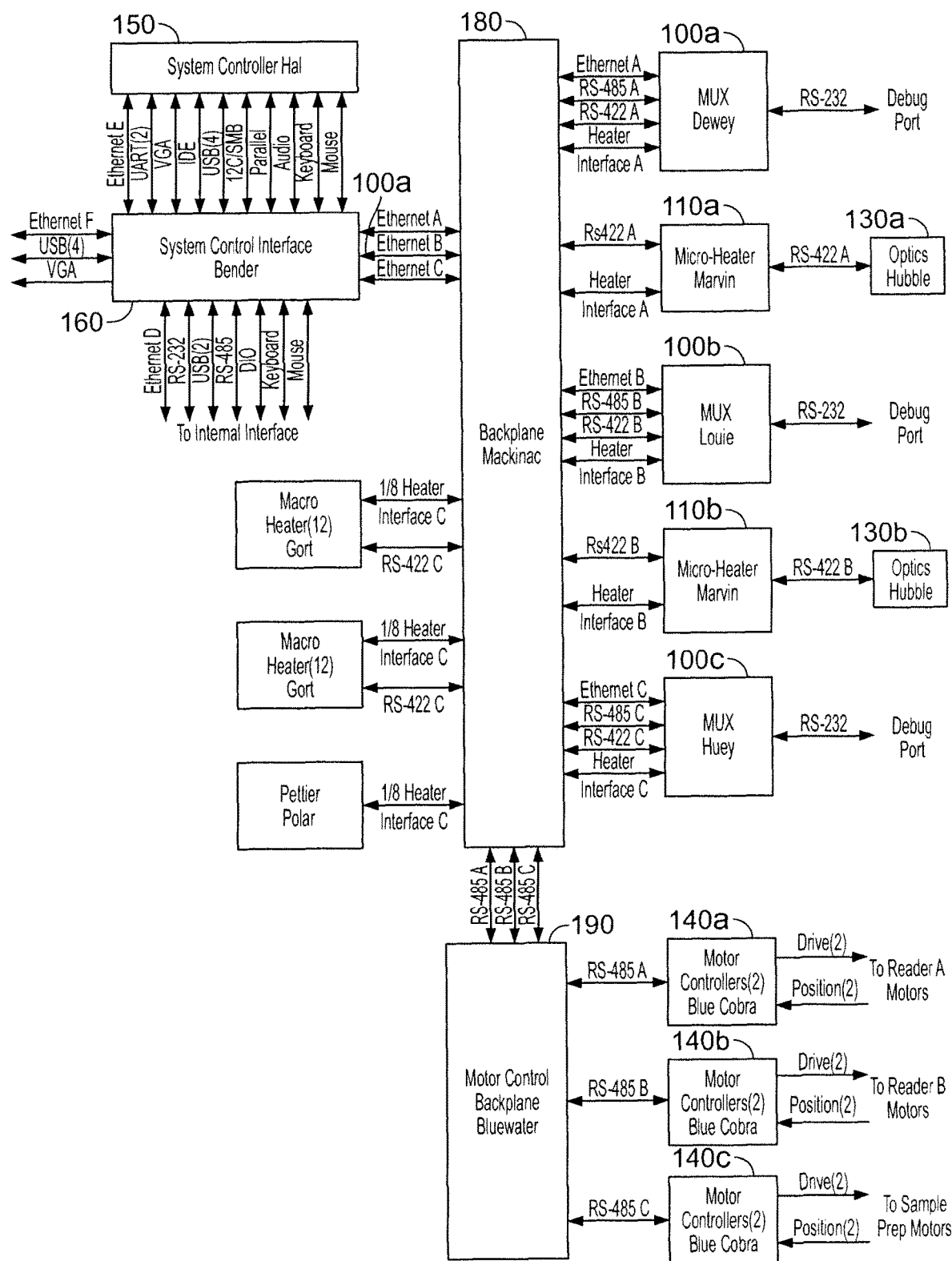


FIG. 86 Electronics Block Diagram

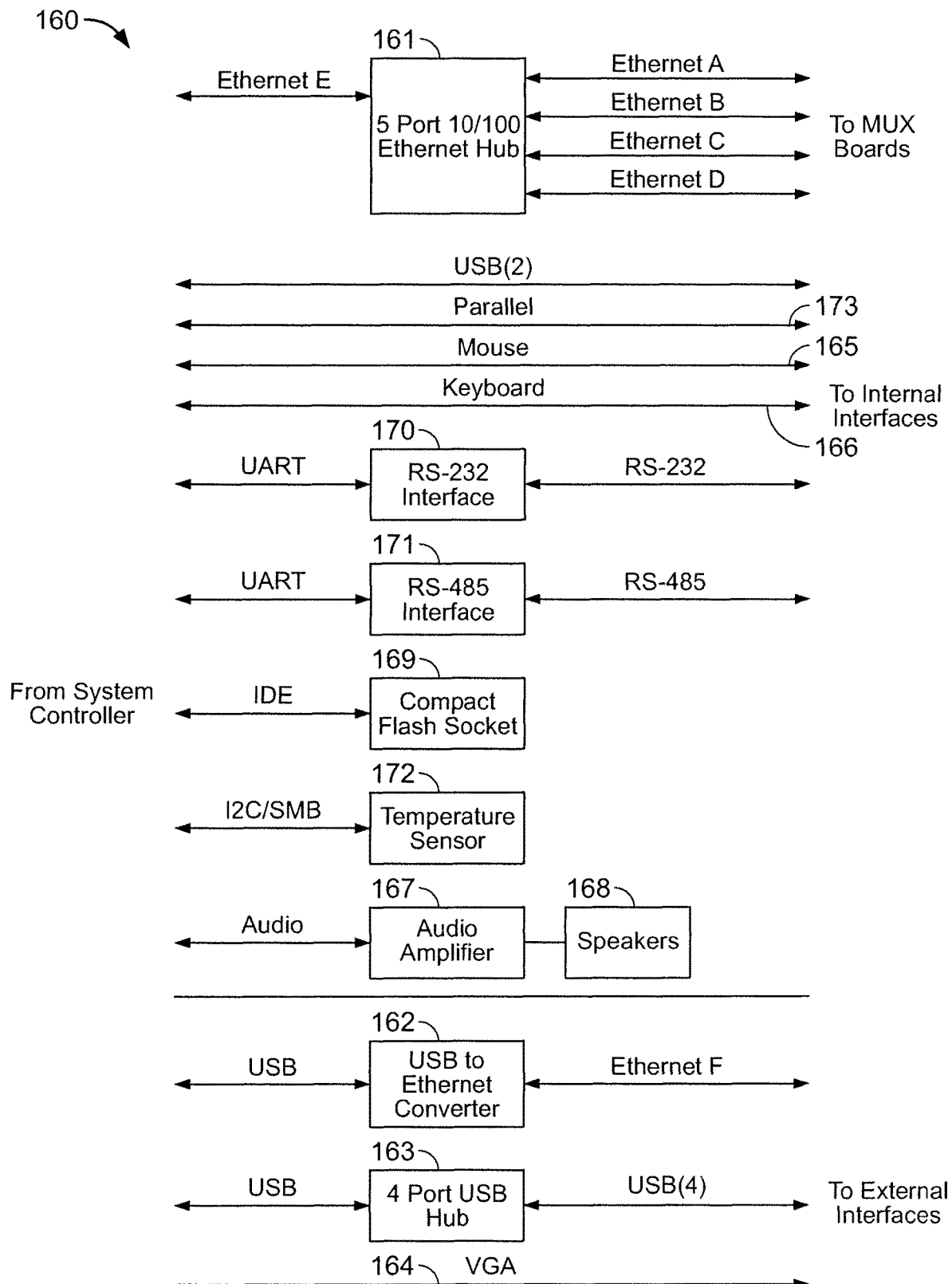


FIG. 87 Processor Base Board Block Diagram

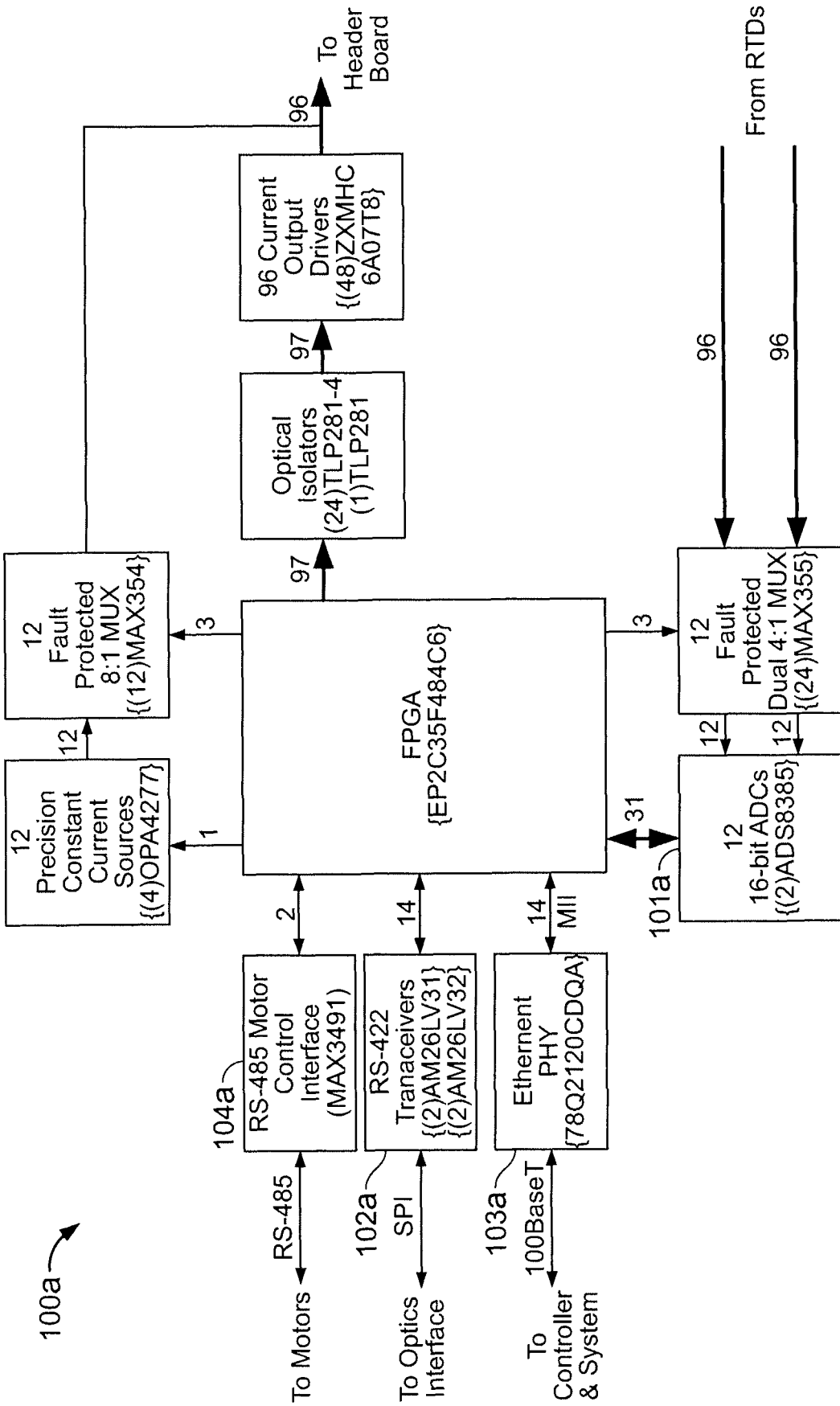


FIG. 88 MUX Board Block Diagram

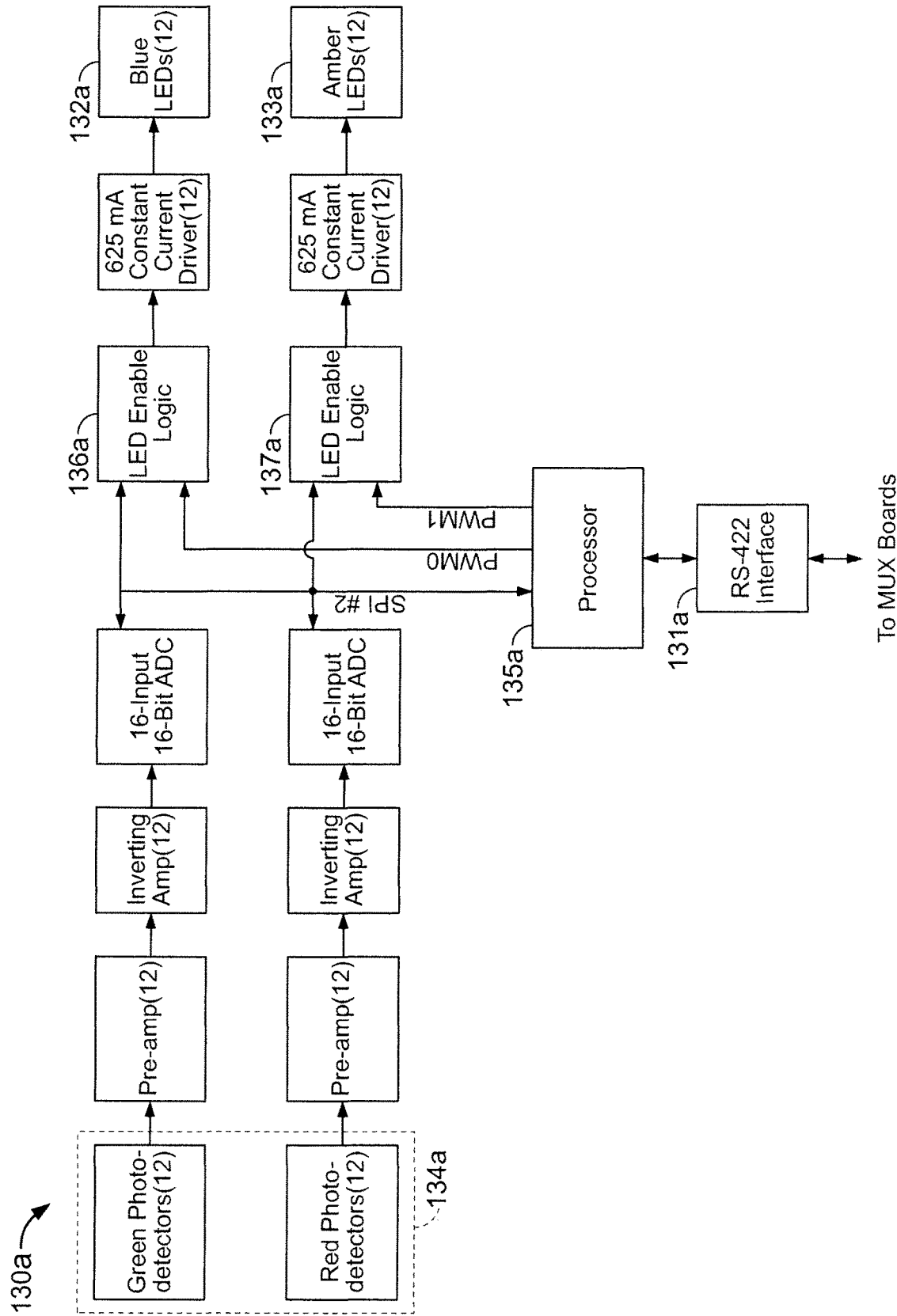


FIG. 89 MUX Board Block Diagram

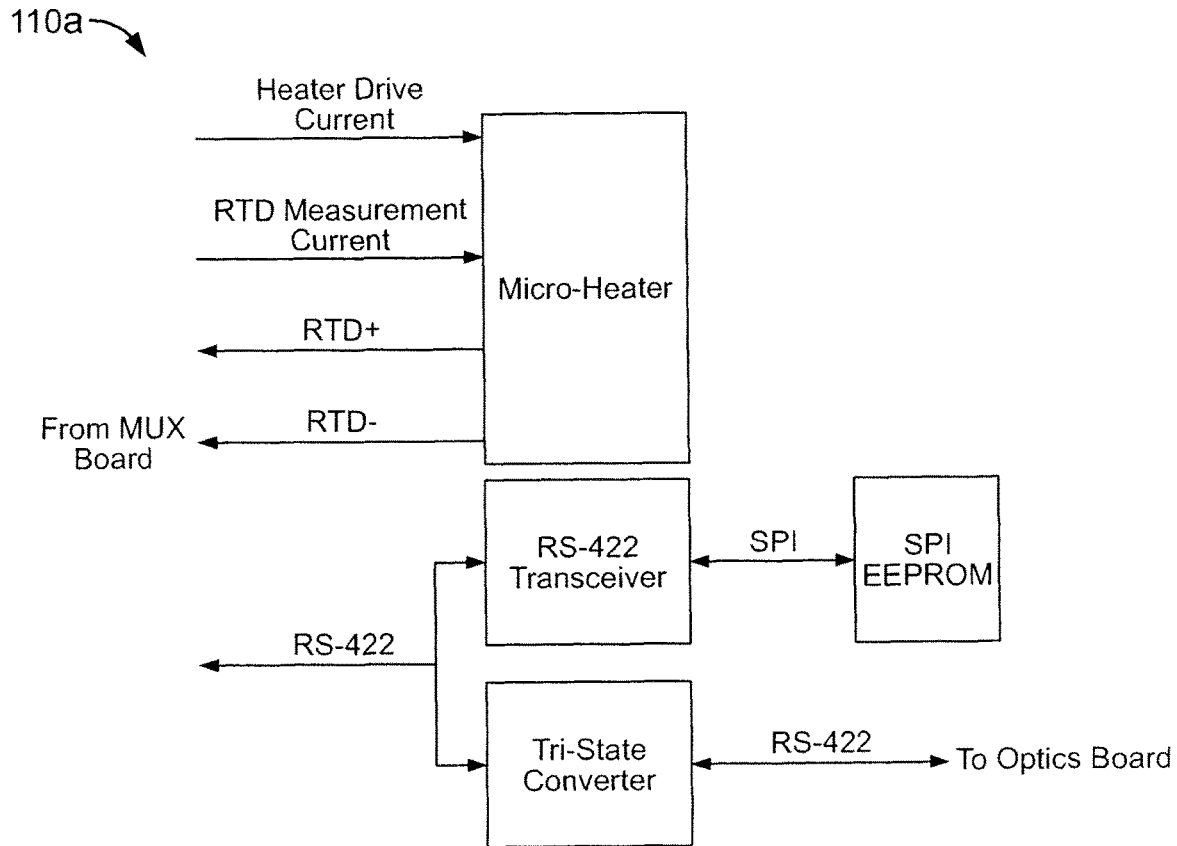


FIG. 90 Micro-Heater Board Block Diagram

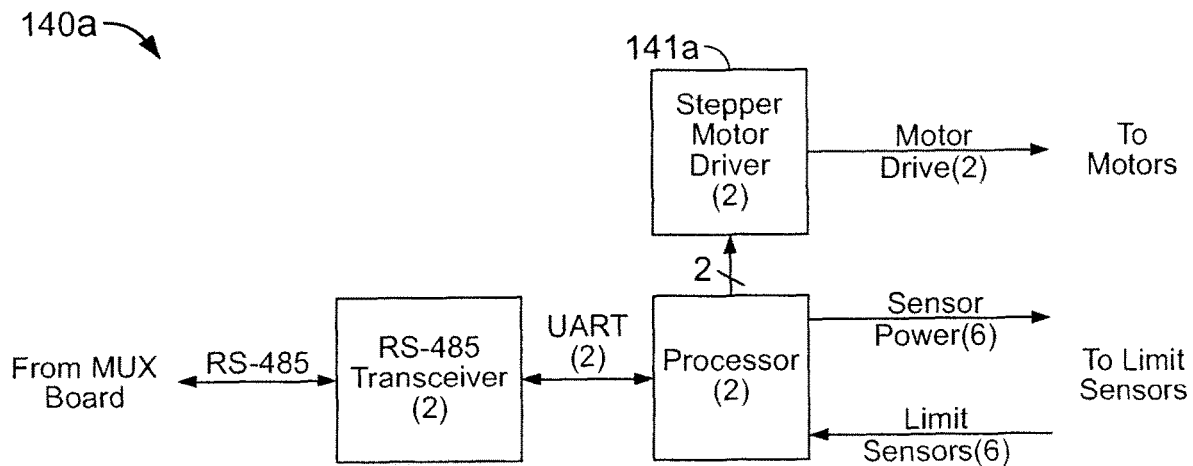


FIG. 91 Motor Control Board Block Diagram

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INTEGRATED APPARATUS FOR PERFORMING NUCLEIC ACID EXTRACTION AND DIAGNOSTIC TESTING ON MULTIPLE BIOLOGICAL SAMPLES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 16/124,672, filed Sep. 7, 2018, which is a continuation of U.S. patent application Ser. No. 14/941,087, filed Nov. 13, 2015 and issued as U.S. Pat. No. 10,071,376 on Sep. 11, 2018, which is a continuation of U.S. patent application Ser. No. 12/218,498, filed Jul. 14, 2008 and issued as U.S. Pat. No. 9,186,677 on Nov. 17, 2015, which claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/959,437, filed Jul. 13, 2007, and is a continuation-in-part of U.S. patent application Ser. No. 11/985,577, filed Nov. 14, 2007 and issued on Aug. 16, 2011 as U.S. Pat. No. 7,998,708. The disclosures of all of the above-referenced prior applications, publications, and patents are considered part of the disclosure of this application, and are incorporated by reference herein in their entirety.

TECHNICAL FIELD

The technology described herein generally relates to systems for extracting polynucleotides from multiple samples, particularly from biological samples, and additionally to systems that subsequently amplify and detect the extracted polynucleotides. The technology more particularly relates to microfluidic systems that carry out PCR on multiple samples of nucleotides of interest within microfluidic channels, and detect those nucleotides.

BACKGROUND

The medical diagnostics industry is a critical element of today's healthcare infrastructure. At present, however, in vitro diagnostic analyses no matter how routine have become a bottleneck in patient care. There are several reasons for this. First, many diagnostic analyses can only be done with highly specialist equipment that is both expensive and only operable by trained clinicians. Such equipment is found in only a few locations—often just one in any given urban area. This means that most hospitals are required to send out samples for analyses to these locations, thereby incurring shipping costs and transportation delays, and possibly even sample loss or mishandling. Second, the equipment in question is typically not available 'on-demand' but instead runs in batches, thereby delaying the processing time for many samples because they must wait for a machine to fill up before they can be run.

Understanding that sample flow breaks down into several key steps, it would be desirable to consider ways to automate as many of these as possible. For example, a biological sample, once extracted from a patient, must be put in a form suitable for a processing regime that typically involves using PCR to amplify a vector (such as a nucleotide) of interest. Once amplified, the presence of a nucleotide of interest from the sample needs to be determined unambiguously. Preparing samples for PCR is currently a time-consuming and labor intensive step, though not one requiring specialist skills, and could usefully be automated. By contrast, steps such as PCR and nucleotide detection (or 'nucleic acid

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testing') have customarily only been within the compass of specially trained individuals having access to specialist equipment.

There is a need for a method and apparatus of carrying out sample preparations on samples in parallel, with or without PCR and detection on the prepared biological samples, and preferably with high throughput, but in a manner that can be done routinely at the point of care, or without needing the sample to be sent out to a specialized facility.

The discussion of the background herein is included to explain the context of the inventions described herein. This is not to be taken as an admission that any of the material referred to was published, known, or part of the common general knowledge as at the priority date of any of the claims.

Throughout the description and claims of the specification the word "comprise" and variations thereof, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

SUMMARY

A diagnostic apparatus, comprising: a first module configured to extract nucleic acid simultaneously from a plurality of nucleic-acid containing samples, wherein the first module comprises: one or more racks, each configured to accept a number of samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain respectively sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample; a magnetic separator configured to move relative to the process chambers of each holder; a heater assembly configured to independently heat each of the process chambers; and a liquid dispenser configured to carry out fluid transfer operations on two or more holders simultaneously; and a second module configured to simultaneously amplify the nucleic acid extracted from the plurality of samples, wherein the second module comprises: one or more bays, each configured to receive a microfluidic cartridge, wherein the cartridge is configured to separately accept and to separately amplify the nucleic acid extracted from multiple samples; and one or more detection systems.

A diagnostic apparatus comprising: one or more racks, on each of which is mounted a number of nucleic acid containing samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain, respectively, sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample; a magnetic separator movable from a first position to a second position adjacent to the process chamber of each of the one or more holders; a heater assembly comprising a number of heater units, each of which is in thermal contact with one of the process chambers; one or more bays, each bay having a shape complementary to a shape of a microfluidic cartridge, wherein the cartridge comprises a number of inlets each of which is in fluid communication with one of a number of channels in which nucleic acid extracted from one of the number of samples is amplified, and wherein the cartridge further comprises one or more windows that permit detection of amplified nucleic acid; a liquid dispenser having one or more dispensing heads, wherein the liquid dispenser is movable from a first position above a first holder to a second position above a second holder, and is movable from the first

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position above the first holder to a different position above the first holder, and is further movable from a position above one of the holders to a position above one of the number of inlets; and one or more detection systems positioned in proximity to the one or more windows.

A diagnostic instrument comprising: a liquid handling unit that extracts nucleic acid from a sample in a unitized reagent strip; a microfluidic cartridge that, in conjunction with a heater element, carries out real-time PCR on nucleic acid extracted from the sample; and a detector that provides a user with a diagnosis of whether the sample contains a nucleotide of interest.

Also described herein are methods of using the diagnostic apparatus, including a method of diagnosing a number of samples in parallel, using the apparatus.

A unitized reagent holder, comprising: a strip, to which is attached: a single process tube; one or more receptacles, each of which holding a reagent selected from the group consisting of: a sample preparation reagent, PCR reagents for a first analyte, and one or more liquid reagents; a waste tube; one or more sockets configured to hold one or more pipette tips; and a pipette tip sheath configured to surround the one or more pipette tips.

A liquid dispenser, comprising: one or more sensors; a manifold; one or more pumps in fluid communication with the manifold; one or more dispense heads in fluid communication with the manifold; a gantry that provides freedom of translational motion in three dimensions; and electrical connections that accept electrical signals from an external controller, wherein the liquid dispenser has no inlet or outlet for fluids, other than through the one or more pumps.

A separator for magnetic particles, comprising: one or more magnets aligned linearly; a motorized shaft upon which the one or more magnets can rise or fall in such a manner that the one or more magnets attains close proximity to one or more receptacles containing magnetic particles; and control circuitry to control motion of the motorized shaft.

An integrated separator and heater, comprising: a heater assembly, wherein the heater assembly comprises a plurality of independently controllable heater units, each of which is configured to accept and to heat a process chamber; one or more magnets aligned linearly; a motorized shaft upon which the one or more magnets can rise or fall in such a manner that the one or more magnets attains close proximity to one or more of the process chambers; and control circuitry to control motion of the motorized shaft and heating of the heater units.

A preparatory apparatus comprising: a first module configured to extract nucleic acid simultaneously from a number of nucleic-acid containing samples, wherein the first module comprises: one or more racks, each configured to accept the number of samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain, respectively, sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample; a magnetic separator configured to move relative to the process chambers of each holder; a heater assembly configured to independently heat each of the process chambers; and a liquid dispenser configured to carry out fluid transfer operations on two or more holders simultaneously; and a second module configured to receive and to store the nucleic acid extracted from the number of samples.

A preparatory apparatus comprising: one or more racks, on each of which is mounted a number of nucleic acid

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containing samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain, respectively, sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample; a magnetic separator movable from a first position to a second position adjacent to the process chambers of each holder; a heater assembly comprising a number of heater units, each of which is in contact with process chamber; a liquid dispenser movable from a first position above a first holder to a second position above a second holder; and a storage compartment having a number of compartments, wherein each compartment stores the nucleic acid extracted from one of the number of samples.

A unitized reagent holder, comprising: a strip, to which is attached: a single process tube; one or more receptacles, each of which holding a reagent selected from the group consisting of: a sample preparation reagent, and one or more liquid reagents; a waste tube; one or more sockets configured to hold one or more pipette tips; and a pipette tip sheath configured to surround the one or more pipette tips.

The present technology additionally includes a process for extracting nucleic acid from multiple samples in parallel, using the apparatus as described herein.

BRIEF DESCRIPTION OF SELECTED DRAWINGS

FIG. 1A show a schematic of a preparatory apparatus; FIG. 1B shows a schematic of a diagnostic apparatus.

FIG. 2 shows a schematic of control circuitry.

FIGS. 3A and 3B show exterior views of an exemplary apparatus.

FIG. 4 shows an exemplary interior view of an apparatus.

FIG. 5 shows perspective views of an exemplary rack for sample holders.

FIG. 6 shows perspective views of the rack of FIG. 5 in conjunction with a heater unit.

FIG. 7 shows a perspective view of an exemplary rack for sample holders.

FIGS. 8A-8K show various views of the rack of FIG. 7.

FIG. 9 shows an area of an apparatus configured to accept a rack of FIG. 7.

FIGS. 10A and 10B show a first exemplary embodiment of a reagent holder having pipette sheath, in perspective view (FIG. 10A) and underside view (FIG. 10B).

FIG. 11 shows an exemplary embodiment of a reagent holder not having a pipette sheath, in perspective view.

FIGS. 12A-12C show a second exemplary embodiment of a reagent holder having a pipette sheath, in perspective view (FIG. 12A) and cross-sectional view (FIG. 12B), and exploded view (FIG. 12C).

FIGS. 13A and 13B show a stellated feature on the interior of a reagent tube, in cross-sectional (FIG. 13A) and plan (FIG. 13B) view.

FIG. 14 shows a sequence of pipetting operations in conjunction with a reagent tube having a stellated feature.

FIG. 15 shows embodiments of a laminated layer.

FIG. 16 shows a sequence of pipetting operations in conjunction with a laminated layer.

FIGS. 17A-17D show an exemplary kit containing holders and reagents.

FIG. 18 shows a liquid dispense head.

FIGS. 19A-19C show a liquid dispense head.

FIG. 20 shows an exemplary distribution manifold.

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FIG. 21 shows a scanning read-head attached to a liquid dispense head.

FIG. 22 shows a barcode scanner in cross-sectional view.

FIG. 23 shows a barcode reader positioned above a microfluidic cartridge.

FIG. 24 shows pipette tip sensors.

FIGS. 25A and 25B show an exemplary device for stripping pipette tip.

FIG. 26 shows a heater unit in perspective and cross-sectional view.

FIG. 27 shows an integrated heater and separator unit in cross-sectional view.

FIG. 28 shows a cartridge auto-loader.

FIG. 29 shows a cartridge stacker.

FIG. 30 shows a cartridge stacker in position to deliver a cartridge to an auto-loader.

FIG. 31 shows a cartridge loading system.

FIG. 32 shows a disposal unit for used cartridges.

FIG. 33 shows a cartridge stacker in full and empty configurations.

FIG. 34 shows a microfluidic cartridge, a read-head, and a cartridge tray.

FIG. 35 shows a cross-section of a pipetting head and a cartridge in position in a microfluidic apparatus.

FIG. 36 shows an exemplary microfluidic cartridge having a 3-layer construction.

FIG. 37 shows a plan of microfluidic circuitry and inlets in an exemplary multi-lane cartridge.

FIG. 38A shows an exemplary multi-lane cartridge.

FIG. 38B shows a portion of an exemplary multi-layer cartridge.

FIGS. 39A, 39B show an exemplary microfluidic network in a lane of a multi-lane cartridge;

FIGS. 40A-40C show diagrams of exemplary microfluidic valves. FIG. 40A additionally shows the valve in an open state, and the valve in a closed state.

FIG. 41 shows a vent.

FIG. 42 shows an exemplary highly-multiplexed microfluidic cartridge;

FIGS. 43-46 show various aspects of exemplary highly multiplexed microfluidic cartridges; and

FIGS. 47A-C show various aspects of a radially configured highly multiplexed microfluidic cartridge.

FIG. 48 shows a view in cross-section of a microfluidic cartridge.

FIGS. 49A, 49B show a PCR reaction chamber and associated heaters.

FIG. 50 shows thermal images of heater circuitry in operation.

FIGS. 51A-51C shows various cut-away sections that can be used to improve cooling rates during PCR thermal cycling.

FIG. 52 shows a plot of temperature against time during a PCR process, as performed on a microfluidic cartridge as described herein.

FIG. 53 shows an assembly process for a cartridge as further described herein.

FIGS. 54A and 54B show exemplary apparatus for carrying out wax deposition.

FIGS. 55A and 55B show exemplary deposition of wax droplets into microfluidic valves.

FIG. 56 shows an overlay of an array of heater elements on an exemplary multi-lane microfluidic cartridge, wherein various microfluidic networks are visible.

FIG. 57 shows a cross-sectional view of an exemplary detector.

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FIG. 58 shows a perspective view of a detector in a read-head.

FIG. 59 shows a cutaway view of an exemplary detector in a read-head.

FIG. 60 shows an exterior view of an exemplary multiplexed read-head with an array of detectors therein.

FIG. 61 shows an cutaway view of an exemplary multiplexed read-head with an array of detectors therein.

FIG. 62 shows a block diagram of exemplary electronic circuitry in conjunction with a detector as described herein.

FIG. 63 shows an exemplary liquid dispensing system.

FIG. 64 shows an exemplary heater/separator.

FIGS. 65A and 65B show exemplary aspects of a computer-based user interface.

FIG. 66 shows schematically layout of components of a preparatory apparatus.

FIG. 67 shows layout of components of an exemplary preparatory apparatus.

FIG. 68 shows schematically layout of components of a diagnostic apparatus.

FIG. 69 shows layout of components of an exemplary diagnostic apparatus.

FIGS. 70 and 71 show exterior and interior of an exemplary diagnostic apparatus.

FIGS. 72A and 72B show a thermocycling unit configured to accept a microfluidic cartridge.

FIG. 73 shows schematically a layout of components of a high-efficiency diagnostic apparatus.

FIG. 74 shows layout of components of an exemplary high-efficiency diagnostic apparatus.

FIG. 75 shows a plan view of a 24-lane microfluidic cartridge.

FIG. 76 shows a perspective view of the cartridge of FIG. 75.

FIG. 77 shows an exploded view of the cartridge of FIG. 75.

FIG. 78 shows an exemplary detection unit.

FIGS. 79A, 79B show cutaway portions of the detection unit of FIG. 78.

FIGS. 80, and 81 show alignment of the detection unit with a microfluidic cartridge.

FIGS. 82 and 83 show exterior and cutaways, respectively, of an optics block.

FIG. 84 shows a Scorpion reaction, schematically.

FIGS. 85A-85C show, schematically, pipette head usage during various preparatory processes.

FIGS. 86-91 show exemplary layouts of electronics control circuitry.

DETAILED DESCRIPTION

Nucleic acid testing (NAT) as used herein is a general term that encompasses both DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid) testing. Exemplary protocols that are specific to RNA and to DNA are described herein. It is to be understood that generalized descriptions where not specific to RNA or to DNA either apply to each equally or can be readily adapted to either with minor variations of the description herein as amenable to one of ordinary skill in the art. It is also to be understood that the terms nucleic acid and polynucleotide may be used interchangeably herein.

The apparatuses as described herein therefore find application to analyzing any nucleic acid containing sample for any purpose, including but not limited to genetic testing, and clinical testing for various infectious diseases in humans. Targets for which clinical assays currently exist, and that may be tested for using the apparatus and methods herein

may be bacterial or viral, and include, but are not limited to: Chlamydia Trachomatis (CT); Neisseria Gonorrhea (GC); Group B Streptococcus; HSV; HSV Typing; CMV; Influenza A & B; MRSA; RSV; TB; Trichomonas; Adenovirus; Bordetella; BK; JC; HHV6; EBV; Enterovirus; and *M. pneumoniae*.

The apparatus herein can be configured to run on a laboratory benchtop, or similar environment, and can test approximately 45 samples per hour when run continuously throughout a normal working day. This number can be increased, according to the number of tests that can be accommodated in a single batch, as will become clear from the description herein. Results from individual raw samples are typically available in less than 1 hour.

Where used herein, the term “module” should be taken to mean an assembly of components, each of which may have separate, distinct and/or independent functions, but which are configured to operate together to produce a desired result or results. It is not required that a every component within a module be directly connected or in direct communication with every other. Furthermore, connectivity amongst the various components may be achieved with the aid of a component, such as a processor, that is external to the module.

Apparatus Overview

An apparatus having various components as further described herein can be configured into at least two formats, preparatory and diagnostic, as shown respectively in FIGS. 1A and 1B. A schematic overview of a preparatory apparatus **981** for carrying out sample preparation as further described herein is shown in FIG. 1A. An overview of a diagnostic apparatus **971** is shown in FIG. 1B. The geometric arrangement of the components of system **971**, **981** shown in FIGS. 1A and 1B is exemplary and not intended to be limiting.

A processor **980**, such as a microprocessor, is configured to control functions of various components of the system as shown, and is thereby in communication with each such component requiring control. It is to be understood that many such control functions can optionally be carried out manually, and not under control of the processor. Furthermore, the order in which the various functions are described, in the following, is not limiting upon the order in which the processor executes instructions when the apparatus is operating. Thus, processor **980** can be configured to receive data about a sample to be analyzed, e.g., from a sample reader **990**, which may be a barcode reader, an optical character reader, or an RFID scanner (radio frequency tag reader). It is also to be understood that, although a single processor **980** is shown as controlling all operations of apparatus **971** and **981**, such operations may be distributed, as convenient, over more than one processor.

Processor **980** can be configured to accept user instructions from an input **984**, where such instructions may include instructions to start analyzing the sample, and choices of operating conditions. Although not shown in FIGS. 1A and 1B, in various embodiments, input **984** can include one or more input devices selected from the group consisting of: a keyboard, a touch-sensitive surface, a microphone, a track-pad, a retinal scanner, a holographic projection of an input device, and a mouse. A suitable input device may further comprise a reader of formatted electronic media, such as, but not limited to, a flash memory card, memory stick, USB-stick, CD, or floppy diskette. An input device may further comprise a security feature such as a fingerprint reader, retinal scanner, magnetic strip reader, or bar-code reader, for ensuring that a user of the system is in fact authorized to do so, according to pre-loaded identifying

characteristics of authorized users. An input device may additionally—and simultaneously—function as an output device for writing data in connection with sample analysis. For example, if an input device is a reader of formatted electronic media, it may also be a writer of such media. Data that may be written to such media by such a device include, but is not limited to, environmental information, such as temperature or humidity, pertaining to an analysis, as well as a diagnostic result, and identifying data for the sample in question.

Processor **980** can be also configured to communicate with a display **982**, so that, for example, information about an analysis is transmitted to the display and thereby communicated to a user of the system. Such information includes but is not limited to: the current status of the apparatus; progress of PCR thermocycling; and a warning message in case of malfunction of either system or cartridge. Additionally, processor **980** may transmit one or more questions to be displayed on display **982** that prompt a user to provide input in response thereto. Thus, in certain embodiments, input **984** and display **982** are integrated with one another.

Processor **980** can be optionally further configured to transmit results of an analysis to an output device such as a printer, a visual display, a display that utilizes a holographic projection, or a speaker, or a combination thereof.

Processor **980** can be still further optionally connected via a communication interface such as a network interface to a computer network **988**. The communication interface can be one or more interfaces selected from the group consisting of: a serial connections, a parallel connection, a wireless network connection, a USB connection, and a wired network connection. Thereby, when the system is suitably addressed on the network, a remote user may access the processor and transmit instructions, input data, or retrieve data, such as may be stored in a memory (not shown) associated with the processor, or on some other computer-readable medium that is in communication with the processor. The interface may also thereby permit extraction of data to a remote location, such as a personal computer, personal digital assistant, or network storage device such as computer server or disk farm. The apparatus may further be configured to permit a user to e-mail results of an analysis directly to some other party, such as a healthcare provider, or a diagnostic facility, or a patient.

Additionally, in various embodiments, the apparatus can further comprise a data storage medium configured to receive data from one or more of the processor, an input device, and a communication interface, the data storage medium being one or more media selected from the group consisting of: a hard disk drive, an optical disk drive, a flash card, and a CD-Rom.

Processor **980** can be further configured to control various aspects of sample preparation and diagnosis, as follows in overview, and as further described in detail herein. In FIGS. 1A and 1B, the apparatus **981** (or **971**) is configured to operate in conjunction with a complementary rack **970**. The rack is itself configured, as further described herein, to receive a number of biological samples **996** in a form suitable for work-up and diagnostic analysis, and a number of holders **972** that are equipped with various reagents, pipette tips and receptacles. The rack is configured so that, during sample work-up, samples are processed in the respective holders, the processing including being subjected, individually, to heating and cooling via heater assembly **977**. The heating functions of the heater assembly can be controlled by the processor **980**. Heater assembly **977** operates in conjunction with a separator **978**, such as a magnetic

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separator, that also can be controlled by processor 980 to move into and out of close proximity to one or more processing chambers associated with the holders 972, wherein particles such as magnetic particles are present.

Liquid dispenser 97, which similarly can be controlled by processor 980, is configured to carry out various suck and dispense operations on respective sample, fluids and reagents in the holders 972, to achieve extraction of nucleic acid from the samples. Liquid dispenser 976 can carry out such operations on multiple holders simultaneously. Sample reader 990 is configured to transmit identifying indicia about the sample, and in some instances the holder, to processor 980. In some embodiments a sample reader is attached to the liquid dispenser and can thereby read indicia about a sample above which the liquid dispenser is situated. In other embodiments the sample reader is not attached to the liquid dispenser and is independently movable, under control of the processor. Liquid dispenser 976 is also configured to take aliquots of fluid containing nucleic acid extracted from one or more samples and direct them to storage area 974, which may be a cooler. Area 974 contains, for example, a PCR tube corresponding to each sample. In other embodiments, there is not a separate Area 974, but a cooler can be configured to cool the one or more holders 972 so that extracted nucleic acid is cooled and stored in situ rather than being transferred to a separate location.

FIG. 1B shows a schematic embodiment of a diagnostic apparatus 971, having elements in common with apparatus 981 FIG. 1A but, in place of a storage area 974, having a receiving bay 992 in which a cartridge 994 is received. The receiving bay is in communication with a heater 998 that itself can be controlled by processor 980 in such a way that specific regions of the cartridge are heated at specific times during analysis. Liquid dispenser 976 is thus configured to take aliquots of fluid containing nucleic acid extracted from one or more samples and direct them to respective inlets in cartridge 994. Cartridge 994 is configured to amplify, such as by carrying out PCR, on the respective nucleic acids. The processor is also configured to control a detector 999 that receives an indication of a diagnosis from the cartridge 994. The diagnosis can be transmitted to the output device 986 and/or the display 982, as described hereinabove.

A suitable processor 980 can be designed and manufactured according to, respectively, design principles and semiconductor processing methods known in the art.

Embodiments of the apparatuses shown in outline in FIGS. 1A and 1B, as with other exemplary embodiments described herein, is advantageous because they do not require locations within the apparatus suitably configured for storage of reagents. Neither do embodiments of the system, or other exemplary embodiments herein, require inlet or outlet parts that are configured to receive reagents from, e.g., externally stored containers such as bottles, canisters, or reservoirs. Therefore, the apparatuses in FIGS. 1A and 1B are self-contained and operate in conjunction with holders 972, wherein the holders are pre-packaged with reagents, such as in locations within it dedicated to reagent storage.

The apparatuses of FIGS. 1A and 1B may be configured to carry out operation in a single location, such as a laboratory setting, or may be portable so that they can accompany, e.g., a physician, or other healthcare professional, who may visit patients at different locations. The apparatuses are typically provided with a power-cord so that they can accept AC power from a mains supply or generator. An optional transformer (not shown) built into each apparatus, or situated externally between a power socket and the

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system, transforms AC input power into a DC output for use by the apparatus. The apparatus may also be configured to operate by using one or more batteries and therefore is also typically equipped with a battery recharging system, and various warning devices that alert a user if battery power is becoming too low to reliably initiate or complete a diagnostic analysis.

The apparatuses of FIGS. 1A and 1B may further be configured, in other embodiments, for multiplexed sample analysis and/or analysis of multiple batches of samples, where, e.g., a single rack holds a single batch of samples. In one such configuration, instances of a system, as outlined in FIG. 1B, accept and to process multiple microfluidic cartridges 994. Each component shown in FIGS. 1A and 1B may therefore be present as many times as there are batches of samples, though the various components may be configured in a common housing.

In still another configuration, a system is configured to accept and to process multiple cartridges, but one or more components in FIGS. 1A and 1B is common to multiple cartridges. For example, a single apparatus may be configured with multiple cartridge receiving bays, but a common processor, detector, and user interface suitably configured to permit concurrent, consecutive, or simultaneous, control of the various cartridges. It is further possible that such an embodiment, also utilizes a single sample reader, and a single output device.

In still another configuration, a system as shown in FIG. 1B is configured to accept a single cartridge, wherein the single cartridge is configured to process more than 1, for example, 2, 3, 4, 5, or 6, samples in parallel, and independently of one another. Exemplary technology for creating cartridges that can handle multiple samples is described elsewhere, e.g., in U.S. application Ser. No. 60/859,284, incorporated herein by reference.

It is further consistent with the present technology that a cartridge can be tagged, e.g., with a molecular bar-code indicative of the sample, to facilitate sample tracking, and to minimize risk of sample mix-up. Methods for such tagging are described elsewhere, e.g., in U.S. patent application Ser. No. 10/360,854, incorporated herein by reference.

Control electronics 840 implemented into apparatus 971 or 981, shown schematically in the block diagram in FIG. 2, can include one or more functions in various embodiments, for example, for main control 900, multiplexing 902, display control 904, detector control 906, and the like. The main control function may serve as the hub of control electronics 840 in the apparatuses of FIGS. 1A and 1B, and can manage communication and control of the various electronic functions. The main control function can also support electrical and communications interface 908 with a user or an output device such as a printer 920, as well as optional diagnostic and safety functions. In conjunction with main control function 900, multiplexer function 902 and control sensor data 914 and output current 916 to help control heater assembly 977. The display control function 904 can control output to and, if applicable, interpret input from touch screen LCD 846, which can thereby provide a graphical interface to the user in certain embodiments. The detector function 906 can be implemented in control electronics 840 using typical control and processing circuitry to collect, digitize, filter, and/or transmit the data from a detector 999 such as one or more fluorescence detectors. Additional functions, not shown in FIG. 2, include but are not limited to control functions for controlling elements in FIGS. 1A and 1B such as a liquid dispense head, a separator, a cooler, and to accept data from a sample reader.

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An exemplary apparatus, having functions according to FIGS. 1A or 1B, is shown in FIGS. 3A and 3B. The exemplary apparatus in FIGS. 3A and 3B has a housing 985, and a cover 987, shown in a closed position in FIG. 3A, and in an open position in FIG. 3B to reveal interior features 995. Cover 987 optionally has a handle 989, shown as oval and raised from the surface of the cover, but which may be other shapes such as square, rectangular, or circular, and which may be recessed in, or flush with, the surface of the cover. Cover 987 is shown as having a hinge, though other configurations such as a sliding cover are possible. Bumper 991 serves to prevent the cover from falling too far backwards and/or provides a point that holds cover 987 steady in an open position. Housing 985 is additionally shown as having one or more communications ports 983, and one or more power ports 993, which may be positioned elsewhere, such as on the rear of the instrument.

The apparatus of FIGS. 1A and 1B may optionally comprise one or more stabilizing feet that cause the body of the device to be elevated above a surface on which system 100 is disposed, thereby permitting ventilation underneath system 100, and also providing a user with an improved ability to lift system 100. There may be 2, 3, 4, 5, or 6, or more feet, depending upon the size of system 100. Such feet are preferably made of rubber, or plastic, or metal, and in some embodiments may elevate the body of system 10 by from about 2 to about 10 mm above a surface on which it is situated.

FIG. 4 shows an exemplary configuration of a portion of an interior of an exemplary apparatus, such as that shown in FIGS. 3A and 3B. In FIG. 4 are shown a rack 970, containing a number of reagent holders 972 and patient samples 996, as well as, in close proximity thereto, a receiving bay 992 having a cartridge 994, for performing PCR on polynucleotides extracted from the sample.

Rock

The apparatus further comprises one or more racks configured to be insertable into, and removable from, the apparatus, each of the racks being further configured to receive a plurality of reagent holders, and to receive a plurality of sample tubes, wherein the reagent holders are in one-to-one correspondence with the sample tubes, and wherein the reagent holders each contain sufficient reagents to extract polynucleotides from a sample and place the polynucleotides into a PCR-ready form. Exemplary reagent holders are further described elsewhere herein.

An apparatus may comprise 1, 2, 3, 4, or 6 racks, and each rack may accept 2, 4, 6, 8, 10, 12, 16, or 20 samples such as in sample tubes 802, and a corresponding number of holders 804, each at least having one or more pipette tips, and one or more containers for reagents.

A rack is typically configured to accept a number of reagent holders 804, such as those further described herein, the rack being configured to hold one or more such holders, either permitting access on a laboratory benchtop to reagents stored in the holders, or situated in a dedicated region of the apparatus permitting the holders to be accessed by one or more other functions of the apparatus, such as automated pipetting, heating of the process tubes, and magnetic separating of affinity beads.

Two perspective views of an exemplary rack 800, configured to accept 12 sample tubes and 12 corresponding reagent holders, in 12 lanes, are shown in FIG. 5. A lane, as used herein in the context of a rack, is a dedicated region of the rack designed to receive a sample tube and correspond-

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ing reagent holder. Two perspective views of the same exemplary rack, in conjunction with a heater unit, are shown in FIG. 6.

Various views of a second exemplary rack 800, also configured to accept 12 sample tubes and 12 reagent holders, are shown in FIG. 7, and FIGS. 8A-8C. Thus, the following views are shown: side plan (FIG. 8A); front plan, showing sample tubes (FIG. 8B); rear plan, showing reagent holders (FIG. 8C); rear elevation, showing reagent holders (FIG. 8D); front elevation, showing sample tubes (FIG. 8E); top, showing insertion of a reagent holder (FIGS. 8F and 8G); top showing slot for inserting a reagent holder (FIG. 8H); top view showing registration of reagent holder (FIG. 8I); close up of rack in state of partial insertion/removal from apparatus (FIG. 8J); and rack held by handle, removed from apparatus (FIG. 8K). A recessed area in a diagnostic or preparatory apparatus, as further described herein, for accepting the exemplary removable rack of FIG. 7 is shown in FIG. 9. Other suitably configured recessed areas for receiving other racks differing in shape, appearance, and form, rather than function, are consistent with the description herein.

The two exemplary racks shown in the figures being non-limiting, general features of racks contemplated herein are now described using the two exemplary racks as illustrated thereof. For example, the embodiments shown here, at least the first lane and the second lane are parallel to one another, a configuration that increases pipetting efficiency. Typically, when parallel to one another, pairs of adjacent sample lanes are separated by 24 mm at their respective midpoints. (Other distances are possible, such as 18 mm apart, or 27 mm apart. The distance between the midpoints is dependent on the pitch of the nozzles in the liquid dispensing head, as further described herein. Keeping the spacing in multiples of 9 mm enables easy loading from the rack into a 96 well plate (where typically wells are spaced apart by 9 mm). Typically, also, the rack is such that plurality of reagent holders in the plurality of lanes are maintained at the same height relative to one another.

The rack is configured to accept a reagent holder in such a way that the reagent holder snaps or locks reversibly into place, and remains steady while reagents are accessed in it, and while the rack is being carried from one place to another or is being inserted into, or removed from, the apparatus. In each embodiment, each of the second locations comprises a mechanical key configured to accept the reagent holder in a single orientation. In FIG. 5, it is shown that the reagent holder(s) slide horizontally into vertically oriented slots, one per holder, located in the rack. In such an embodiment, the edge of a connecting member on the holder engages with a complementary groove in the upper portion of a slot. In FIGS. 8F, 8G, and 8I, it is shown that the reagent holder(s) can engage with the rack via a mechanical key that keeps the holders steady and in place. For example, the mechanical key can comprise a raised or recessed portion that, when engaging with a complementary portion of the reagent holder, permits the reagent holder to snap into the second location. It can also be seen in the embodiments shown that the reagent holder has a first end and a second end, and the mechanical key comprises a first feature configured to engage with the first end, and a second feature configured to engage with the second end in such a way that a reagent holder cannot be inserted the wrong way around.

In certain embodiments the reagent holders each lock into place in the rack, such as with a cam locking mechanism that is recognized as locked audibly and/or physically, or such as with a mechanical key. The rack can be configured so that

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the holders, when positioned in it, are aligned for proper pipette tip pick-up using a liquid dispenser as further described herein. Furthermore, the second location of each lane can be deep enough to accommodate one or more pipette tips, such as contained in a pipette tip sheath.

In certain embodiments, the rack is configured to accept the samples in individual sample tubes **802**, each mounted adjacent to a corresponding holder **804**, for example on one side of rack **800**. The sample tubes can be accessible to a sample identification verifier such as a bar code reader, as further described herein. In FIG. **5**, a sample tube is held at its bottom by a cylindrical receiving member. In FIG. **7**, it is shown that a sample tube can be held at both its top and bottom, such as by a recessed portion **803** configured to receive a bottom of a sample tube, and an aperture **805** configured to hold an upper portion of the sample tube. The aperture can be a ring or an open loop, or a hole in a metal sheet. The recessed portion can be as in FIG. **7**, wherein it is an angled sheet of metal housing having a hole large enough to accommodate a sample tube.

The rack can be designed so that it can be easily removed from the apparatus and carried to and from the laboratory environment external to the apparatus, such as a bench, and the apparatus, for example, to permit easy loading of the sample tube(s) and the reagent holder(s) into the rack. In certain embodiments, the rack is designed to be stable on a horizontal surface, and not easily toppled over during carriage, and, to this end, the rack has one or more (such as 2, 3, 4, 6, 8) feet **809**. In certain embodiments, the rack has a handle **806** to ease lifting and moving, and as shown in FIG. **5**, the handle can be locked into a vertical position, during carriage, also to reduce risk of the rack being toppled over. The handle can optionally have a soft grip **808** in its middle. In the embodiment of FIG. **7**, the carrying handle is positioned about an axis displaced from an axis passing through the center of gravity of the rack when loaded, and is free to fall to a position flush with an upper surface of the rack, under its own weight.

The embodiment of FIG. **5** has a metallic base member **810** having 4 feet **811** that also serve as position locations when inserting the rack into the dedicated portion of the apparatus. The handle is attached to the base member. The portion of the rack **812** that accepts the samples and holders can be made of plastic, and comprises 12 slots, and may be disposable.

In the embodiment of FIG. **7**, the rack comprises a housing, a plurality of lanes in the housing, and wherein each lane of the plurality of lanes comprises: a first location configured to accept a sample tube; and a second location, configured to accept a reagent holder; and a registration member complementary to a receiving bay of a diagnostic apparatus. Typically, the housing is made of a metal, such as aluminum, that is both light but also can be machined to high tolerance and is sturdy enough to ensure that the rack remains stable when located in the diagnostic apparatus. The registration member in FIG. **7** comprises four (4) tight tolerance pegs **815**, located one per corner of the rack. Such pegs are such that they fit snugly and tightly into complementary holes in the receiving bay of the apparatus and thereby stabilize the rack. Other embodiments having, for example, 2, or 3, or greater than 4 such pegs are consistent with the embodiments herein.

In particular, the housing in the embodiment of FIG. **7** comprises a horizontal member **821**, and two or more vertical members **822** connected to the horizontal member, and is such that the second location of each respective lane is a recessed portion within the horizontal member. The two

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or more vertical members **809** in the embodiment of FIG. **7** are configured to permit the rack to free stand thereon. The housing may further comprise two or more feet or runners, attached symmetrically to the first and second vertical members and giving the rack additional stability when positioned on a laboratory bench top.

Furthermore, in the embodiment of FIG. **7**, the housing further comprises a plurality of spacer members **825**, each of which is disposed between a pair of adjacent lanes. Optionally, such spacer members may be disposed vertically between the lanes.

Although not shown in the FIGs., a rack can further comprise a lane identifier associated with each lane. A lane identifier may be a permanent or temporary marking such as a unique number or letter, or can be an RFID, or bar-code, or may be a colored tag unique to a particular lane.

A rack is configured so that it can be easily placed at the appropriate location in the instrument and gives the user positive feedback, such as audibly or physically, that it is placed correctly. In certain embodiments, the rack can be locked into position. It is desirable that the rack be positioned correctly, and not permitted to move thereafter, so that movement of the liquid dispenser will not be compromised during liquid handling operations. The rack therefore has a registration member to ensure proper positioning. In the embodiment of FIG. **7**, the registration member comprises two or more positioning pins configured to ensure that the rack can only be placed in the diagnostic apparatus in a single orientation; and provide stability for the rack when placed in the diagnostic apparatus. The embodiment of FIG. **7** has, optionally, a sensor actuator **817** configured to indicate proper placement of the rack in the diagnostic apparatus. Such a sensor may communicate with a processor **980** to provide the user with a warning, such as an audible warning, or a visual warning communicated via an interface, if the rack is not seated correctly. It may also be configured to prevent a sample preparation process from initiating or continuing if a seating error is detected.

In certain embodiments, the interior of the rack around the location of process tubes in the various holders is configured to have clearance for a heater assembly and/or a magnetic separator as further described herein. For example, the rack is configured so the process chambers on the individual holders are accepted by heater units in a heater assembly as further described herein.

Having a removable rack enables a user to keep a next rack loaded with samples and in line while a previous rack of samples is being prepared by the apparatus, so that the apparatus usage time is maximized.

The rack can also be conveniently cleaned outside of the instrument in case of any sample spills over it or just as a routine maintenance of laboratory wares.

In certain embodiments the racks have one or more disposable parts.

Holder

FIGS. **10A** and **10B** show views of an exemplary holder **501** as further described herein. FIG. **11** shows a plan view of another exemplary holder **502**, as further described herein. FIG. **12A** shows an exemplary holder **503** in perspective view, and FIG. **12B** shows the same holder in cross-sectional view. FIG. **12C** shows an exploded view of the same holder as in FIGS. **12A** and **12B**. All of these exemplary holders, as well as others consistent with the written description herein though not shown as specific embodiments, are now described.

The exemplary holders shown in FIGS. **10A**, **10B**, **11**, **12A**, **12B**, and **12C** can each be referred to as a “unitized

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disposable strip”, or a “unitized strip”, because they are intended to be used as a single unit that is configured to hold all of the reagents and receptacles necessary to perform a sample preparation, and because they are laid out in a strip format. It is consistent with the description herein, though, that other geometric arrangements of the various receptacles are contemplated, so that the description is not limited to a linear, or strip, arrangement, but can include a circular or grid arrangement.

Some of the reagents contained in the holder are provided as liquids, and others may be provided as solids. In some embodiments, a different type of container or tube is used to store liquids from those that store the solids.

The holder can be disposable, such as intended for a single use, following which it is discarded.

The holder is typically made of a plastic such as polypropylene. The plastic is such that it has some flexibility to facilitate placement into a rack, as further described herein. The plastic is typically rigid, however, so that the holder will not significantly sag or flex under its own weight and will not easily deform during routine handling and transport, and thus will not permit reagents to leak out from it.

The holder comprises a connecting member **510** having one or more characteristics as follows. Connecting member **510** serves to connect various components of the holder together. Connecting member **510** has an upper side **512** and, opposed to the upper side, an underside **514**. In FIG. **10B**, a view of underside **514** is shown, having various struts **597** connecting a rim of the connecting member with variously the sockets, process tube, and reagent tubes. Struts **597** are optional, and may be omitted all or in part, or may be substituted by, in all or in part, other pieces that keep the holder together.

The holder is configured to comprise: a process tube **520** affixed to the connecting member and having an aperture **522** located in the connecting member; at least one socket **530**, located in the connecting member, the socket configured to accept a disposable pipette tip **580**; two or more reagent tubes **540** disposed on the underside of the connecting member, each of the reagent tubes having an inlet aperture **542** located in the connecting member; and one or more receptacles **550**, located in the connecting member, wherein the one or more receptacles are each configured to receive a complementary container such as a reagent tube (not shown) inserted from the upper side **512** of the connecting member.

The holder is typically such that the connecting member, process tube, and the two or more reagent tubes are made from a single piece, such as a piece of polypropylene.

The holder is also typically such that at least the process tube, and the two or more reagent tubes are translucent.

The one or more receptacles **550** are configured to accept reagent tubes that contain, respectively, sufficient quantities of one or more reagents typically in solid form, such as in lyophilized form, for carrying out extraction of nucleic acid from a sample that is associated with the holder. The receptacles can be all of the same size and shape, or may be of different sizes and shapes from one another. Receptacles **550** are shown as having open bottoms, but are not limited to such topologies, and may be closed other than the inlet **552** in the upper side of connecting member **510**. Preferably the receptacles **550** are configured to accept commonly used containers in the field of laboratory analysis, or containers suitably configured for use with the holder herein. The containers are typically stored separately from the holders to facilitate sample handling, since solid reagents normally

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require different storage conditions from liquid reagents. In particular many solid reagents may be extremely moisture sensitive.

The snapped-in reagent tubes containing different reagents may be of different colors, or color-coded for easy identification by the user. For example they may be made of different color material, such as tinted plastic, or may have some kind of identifying tag on them, such as a color stripe or dot. They may also have a label printed on the side, and/or may have an identifier such as a barcode on the sealing layer on the top.

The containers **554** received by the receptacles **550** may alternatively be an integrated part of the holder and may be the same type of container as the waste chamber and/or the reagent tube(s), or may be different therefrom.

In one embodiment, the containers **554** containing lyophilized reagents, disposed in the receptacles **550** (shown, e.g., in FIGS. **12A** and **12C**), are 0.3 ml tubes that have been further configured to have a star pattern (see FIGS. **13A** and **13B**) on their respective bottom interior surfaces. This is so that when a fluid has been added to the lyophilized reagents (which are dry in the initial package), a pipette tip can be bottomed out in the tube and still be able to withdraw almost the entire fluid from the tube, as shown in FIG. **14**, during the process of nucleic acid extraction. The design of the star-pattern is further described elsewhere herein.

The reagent tubes, such as containing the lyophilized reagents, can be sealed across their tops by a metal foil, such as an aluminum foil, with no plastic lining layer, as further described herein.

The embodiments **501**, **502**, and **503** are shown configured with a waste chamber **560**, having an inlet aperture **562** in the upper side of the connecting member. Waste chamber **560** is optional and, in embodiments where it is present, is configured to receive spent liquid reagents. In other embodiments, where it is not present, spent liquid reagents can be transferred to and disposed of at a location outside of the holder, such as, for example, a sample tube that contained the original sample whose contents are being analyzed. Waste chamber **560** is shown as part of an assembly comprising additionally two or more reagent tubes **540**. It would be understood that such an arrangement is done for convenience, e.g., of manufacture; other locations of the waste chamber are possible, as are embodiments in which the waste chamber is adjacent a reagent tube, but not connected to it other than via the connecting member.

The holder is typically such that the connecting member, process tube, the two or more reagent tubes, and the waste chamber (if present) are made from a single piece, made from a material such as polypropylene.

The embodiments **501** and **503** are shown having a pipette sheath **570**. This is an optional component of the holders described herein. It may be permanently or removably affixed to connecting member **510**, or may be formed, e.g., moulded, as a part of a single piece assembly for the holder. For example, exploded view of holder **503** in FIG. **12C** shows lug-like attachments **574** on the upper surface of a removable pipette sheath **570** that engage with complementary recessed portions or holes in the underside **514** of connecting member **510**. Other configurations of attachment are possible. Pipette sheath **570** is typically configured to surround the at least one socket and a tip and lower portion of a pipette tip when the pipette tip is stationed in the at least one socket. In some embodiments, the at least one socket comprises four sockets. In some embodiments the at least one socket comprises two, three, five, or six sockets.

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Pipette sheath **570** typically is configured to have a bottom **576** and a walled portion **578** disposed between the bottom and the connecting member. Pipette sheath **570** may additionally and optionally have one or more cut-out portions **572** in the wall **578**, or in the bottom **576**. Such cutouts provide ventilation for the pipette tips and also reduce the total amount of material used in manufacture of the holder. Embodiment **503** has a pipette sheath with no such cutouts. In embodiment **501**, such as cutout is shown as an isosceles triangle in the upper portion of the sheath; a similar shaped cutout may be found at a corresponding position in the opposite side of the sheath, obscured from view in FIG. **10A**. Other cutouts could have other triangular forms, circular, oval, square, rectangular, or other polygonal or irregular shapes, and be several, such as many, in number. The wall **578** of pipette sheath **570** may also have a mesh or frame like structure having fenestrations or interstices. In embodiments having a pipette sheath, a purpose of the sheath is to catch drips from used pipette tips, and thereby to prevent cross-sample contamination, from use of one holder to another in a similar location, and/or to any supporting rack in which the holder is situated. Typically, then, the bottom **576** is solid and bowl-shaped (concave) so that drips are retained within it. An embodiment such as **502**, having no pipette sheath, could utilize, e.g., a drip tray or a drainage outlet, suitably placed beneath pipette tips located in the one or more sockets, for the same purpose. In addition to catching drips, the pipette tip sheath prevents or inhibits the tips of other reagent holders—such as those that are situated adjacent to the one in question in a rack as further described herein—from touching each other when the tips are picked up and/or dropped off before or after some liquid processing step. Contact between tips in adjacent holders is generally not intended by, for example, an automated dispensing head that controls sample processing on holders in parallel, but the pipette tips being long can easily touch a tip in a nearby strip if the angle when dropping off of the tip deviates slightly from vertical.

The holders of embodiments **501**, **502**, and **503**, all have a connecting member that is configured so that the at least one socket, the one or more receptacles, and the respective apertures of the process tube, and the two or more reagent tubes, are all arranged linearly with respect to one another (i.e., their midpoints lie on the same axis). However, the holders herein are not limited to particular configurations of receptacles, waster chamber, process tube, sockets, and reagent tubes. For example, a holder may be made shorter, if some apertures are staggered with respect to one another and occupy 'off-axis' positions. The various receptacles, etc., also do not need to occupy the same positions with respect to one another as is shown in FIGS. **12A** and **12B**, wherein the process tube is disposed approximately near the middle of the holder, liquid reagents are stored in receptacles mounted on one side of the process tube, and receptacles holding solid reagents are mounted on the other side of the process tube. Thus, in FIGS. **10A**, **10B**, and **11**, the process tube is on one end of the connecting member, and the pipette sheath is at the other end, adjacent to, in an interior position, a waster chamber and two or more reagent tubes. Still other dispositions are possible, such as mounting the process tube on one end of the holder, mounting the process tube adjacent the pipette tips and pipette tip sheath (as further described herein), and mounting the waste tube adjacent the process tube. It would be understood that alternative configurations of the various parts of the holder give rise only to variations of form and can be accommodated within other variations of the apparatus as described, including but not limited to

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alternative instruction sets for a liquid dispensing pipette head, heater assembly, end magnetic separator, as further described herein.

Process tube **520** can also be a snap-in tube, rather than being part of an integrated piece. Process tube **520** is typically used for various mixing and reacting processes that occur during sample preparation. For example, cell lysis can occur in process tube **520**, as can extraction of nucleic acids. Process tube **520** is then advantageously positioned in a location that minimizes, overall, pipette head moving operations involved with transferring liquids to process tube **520**.

Reagent tubes **540** are typically configured to hold liquid reagents, one per tube. For example, in embodiments **501**, **502**, and **503**, three reagent tubes are shown, containing respectively wash buffer, release buffer, and neutralization buffer, each of which is used in a sample preparation protocol.

Reagent tubes **540** that hold liquids or liquid reagents can be sealed with a laminate structure **598**. The laminate structure typically has a heat seal layer, a plastic layer such as a layer of polypropylene, and a layer of metal such as aluminum foil, wherein the heat seal layer is adjacent the one or more reagent tubes. The additional plastic film that is used in a laminate for receptacles that contain liquid reagents is typically to prevent liquid from contacting the aluminum.

Two embodiments of a laminate structure, differing in their layer structures, are shown in FIG. **15**. In both embodiments, the heat seal layer **602**, for example made of a laquer or other such polymer with a low melting point, is at the bottom, adjacent to the top of the holder, when so applied. The plastic layer **604** is typically on top of the heat seal layer, and is typically made of polypropylene, having a thickness in the range 10-50 microns. The metal layer **608** is typically on top of the plastic layer and may be a layer of Al foil bended to the plastic layer with a layer of adhesive **606**, as in the first embodiment in FIG. **15**, or may be a layer of metal that is evaporated or sputtered into place directly on to the plastic layer. Exemplary thicknesses for the respective layers are shown in FIG. **15**, wherein it is to be understood that variations of up to a factor of 2 in thickness are consistent with the technology herein. In particular, the aluminum foil is 0.1-15 microns thick, and the polymer layer is 15-25 microns thick in one embodiment. In another embodiment, the aluminum is 0.1-1 microns thick, and the polymer layer is 25-30 microns thick.

The laminates deployed herein make longer term storage easier because the holder includes the presence of sealed lyophilized reagents as well as liquids sealed in close proximity, which is normally hard to achieve.

In one embodiment, the tops of the reagent tubes have beveled edges so that when an aluminum foil is heat bonded to the top, the plastic melt does not extend beyond the rim of the tube. This is advantageous because, if the plastic melt reduces the inner diameter of the tube, it will cause interference with the pipette top during operation. In other embodiments, a raised flat portion **599** facilitates application and removal of laminate **598**. Raised surface **599**, on the upper side of the connecting member, and surrounding the inlet apertures to the reagent tubes and, optionally, the waste chamber, is an optional feature of the holder.

The manner in which liquid is pipetted out is such that a pipette tip piercing through the foil rips through without creating a seal around the pipette tip, as in FIG. **16**. Such a seal around the tip during pipetting would be disadvantageous because a certain amount of air flow is desirable for the pipetting operation. In this instance, a seal is not created

because the laminate structure causes the pierced foil to stay in the position initially adopted when it is pierced. The upper five panels in FIG. 16 illustrate the pipetting of a reagent out from a reagent tube sealed with a laminate as further described herein. At A, the pipette tip is positioned approximately centrally above the reagent tube that contains reagent 707. At B, the pipette tip is lowered, usually controllably lowered, into the reagent tube, and in so doing pierces the foil 598. The exploded view of this area shows the edge of the pierced laminate to be in contact with the pipette tip at the widest portion at which it penetrates the reagent tube. At C, the pipette tip is withdrawn slightly, maintaining the tip within the bulk of the reagent 707. The exploded view shows that the pierced foil has retained the configuration that it adopted when it was pierced and the pipette tip descended to its deepest position within the reagent tube. At D, the pipette tip sucks up reagent 707, possibly altering its height as more and more older people undergo such tests. At E, the pipette tip is removed entirely from the reagent tube.

The materials of the various tubes and chambers may be configured to have at least an interior surface smoothness and surface coating to reduce binding of DNA and other macromolecules thereto. Binding of DNA is unwanted because of the reduced sensitivity that is likely to result in subsequent detection and analysis of the DNA that is not trapped on the surface of the holder.

The process tube also may have a low binding surface, and allows magnetic beads to slide up and down the inside wall easily without sticking to it. Moreover, it has a hydrophobic surface coating enabling low suction of fluid and hence low binding of nucleic acids and other molecules.

In some embodiments, the holder comprises a registration member such as a mechanical key. Typically such a key is part of the connecting member 510. A mechanical key ensures that the holder is accepted by a complementary member in, for example, a supporting rack or a receiving bay of an apparatus that controls pipetting operations on reagents in the holder. A mechanical key is normally a particular-shaped cut-out that matches a corresponding cutout or protrusion in a receiving apparatus. Thus, embodiment 501 has a mechanical key 592 that comprises a pair of rectangular-shaped cut-outs on one end of the connecting member. This feature as shown additionally provides for a tab by which a user may gain a suitable purchase when inserting and removing the holder into a rack or another apparatus. Embodiments 501 and 502 also have a mechanical key 590 at the other end of connecting member 510. Key 590 is an angled cutout that eases insertion of the holder into a rack, as well as ensures a good registration therein when abutting a complementary angled cut out in a recessed area configured to receive the holder. Other variations of a mechanical key are, of course, consistent with the description herein, for example, curved cutouts, or various combinations of notches or protrusions all would facilitate secure registration of the holder.

In some embodiments, not shown in FIGS. 10A, 10B, 11, or 12A-C, the holder further comprises an identifier affixed to the connecting member. The identifier may be a label, such as a writable label, a bar-code, a 2-dimensional bar-code, or an RFID tag. The identifier can be, e.g., for the purpose of revealing quickly what combination of reagents is present in the holder and, thus, for what type of sample preparation protocol it is intended. The identifier may also indicate the batch from which the holder was made, for quality control or record-keeping purposes. The identifier may also permit a user to match a particular holder with a particular sample.

It should also be considered consistent with the description herein that a holder additionally can be configured to accept a sample, such as in a sample tube. Thus, in embodiments described elsewhere herein, a rack accepts a number of sample tubes and a number of corresponding holders in such a manner that the sample tubes and holders can be separately and independently loaded from one another. Nevertheless, in other embodiments, a holder can be configured to also accept a sample, for example in a sample tube. And thus, a complementary rack is configured to accept a number of holders, wherein each holder has a sample as well as reagents and other items. In such an embodiment, the holder is configured so that the sample is accessible to a sample identification verifier.

Kits

The holder described herein may be provided in a sealed pouch, to reduce the chance of air and moisture coming into contact with the reagents in the holder. Such a sealed pouch may contain one or more of the holders described herein, such as 2, 4, 6, 8, 10, 12, 16, 20, or 24 holders.

The holder may also be provided as part of a kit for carrying out sample preparation, wherein the kit comprises a first pouch containing one or more of the holders described herein, each of the holders configured with liquid reagents for, e.g., lysis, wash, and release, and a second pouch, having an inert atmosphere inside, and one or more reagent tubes containing lyophilized PCR reagents, as shown in FIG. 17. Such a kit may also be configured to provide for analysis of multiple samples, and contain sufficient PCR reagents (or other amplification reagents, such as for RT-PCR, transcription mediated amplification, strand displacement amplification, NASBA, helicase dependent amplification, and other familiar to one of ordinary skill in the art, and others described herein) to process such samples, and a number of individual holders such as 2, 4, 6, 8, 10, 12, 16, 20, or 24 holders.

Reagent Tubes

As referenced elsewhere herein, the containers 554 that contain lyophilized reagents are 0.3 ml tubes that have been further configured to have a star-shaped—or stellated—pattern (see FIGS. 13A and 13B) on their respective bottom interior surfaces. Still other tubes for use herein, as well as for other uses not herein described, can be similarly configured. Thus, for example, the benefits afforded by the star-shaped pattern also accrue to reagent tubes that contain liquid samples that are directly pipetted out of the tubes (as well as to those tubes that initially hold solids that are constituted into liquid form prior to pipetting). Other size tubes that would benefit from such a star-shaped pattern have sizes in the range 0.1 ml to 0.65 ml, for example.

The star-shaped pattern ensures that when a fluid is withdrawn from the tube, a pipette tip can be bottomed out in the tube and still be able to withdraw the entire, or almost the entire fluid from the tube, as shown in FIG. 14. This is important because, when working with such small volumes, and when target DNA can be present in very few copies, sample loss due to imperfections of pipetting is to be minimized to every extent possible.

The design of the star shaped pattern is important, especially when using for recovery of DNA/RNA present in very small numbers in the clinical sample. The stellated pattern should enable pipetting of most of the liquid (residual volume < 1 microliter) when used with a pipette bottomed out with the bottom of the tube. Additionally, the stellated pattern should be designed to minimize surface area as well as dead-end grooves that tend to have two undesirable

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effects—to trap liquid as well as to increase undesirable retention of polynucleotides by adsorption.

FIG. 14 is now described, as follows. FIG. 14 has a number of panels, A-G, each representing, in sequence, a stage in a pipetting operation. At A, a pipette tip 2210, containing a liquid 2211 (such as a buffer solution), is positioned directly or approximately above the center of reagent tube 2200. The tube contains a number of lyophilized pellets 2212, and is sealed by a layer 2214, such as of foil. The foil may be heat-sealed on to the top of the tube. Although a laminate layer, as further described herein, can be placed on the reagent tube, typically a layer of aluminum foil is adequate, where the tube contents are solid, e.g., lyophilized, reagents. In some embodiments, the top of the reagent tube has chamfer edges to reduce expansion of the top rim of the tube during heat sealing of a foil on the top of the tube. The tube may further comprise an identifiable code, such as a 1-D or a 2-D bar-code on the top. Such a code is useful for identifying the composition of the reagents stored within, and/or a batch number for the preparation thereof, and/or an expiry date. The code may be printed on with, for example, an inkjet or transfer printer.

Stellated pattern 2203 on the bottom interior surface of the tube 2200 is shown. At B, the pipette tip is lowered, piercing seal 2214, and brought into a position above the particles 2212. At C the liquid 2211 is discharged from the pipette tip on to the particles, dissolving the same, as shown at D. After the particles are fully dissolved, forming a solution 2218, the pipette tip is lowered to a position where it is in contact with the stellated pattern 2203. At E, the pipette tip is caused to suck up the solution 2218, and at F, the tip may optionally discharge the solution back into the tube. Steps E and F may be repeated, as desired, to facilitate dissolution and mixing of the lyophilized components into solution. At step G, after sucking up as much of the solution 2218 as is practicable into the pipette tip, the pipette tip is withdrawn from the tube. Ideally, 100% by volume of the solution 2218 is drawn up into the pipette tip at G. In other embodiments, and depending upon the nature of solution 2218, at least 99% by volume of the solution is drawn up. In still other embodiments, at least 98%, at least 97%, at least 96%, at least 95%, and at least 90% by volume of the solution is drawn up.

The design of the stellated or star-shaped pattern can be optimized to maximize the flow rate of liquid through the gaps in-between a bottomed out pipette, such as a p1000 pipette, and the star pattern, and is further described in U.S. provisional patent application Ser. No. 60/959,437, filed Jul. 13, 2007, incorporated herein by reference. It would be understood that, although the description herein pertains to pipettes and pipette tips typically used in sample preparation of biological samples, the principles and detailed aspects of the design are as applicable to other types of pipette and pipette tip, and may be so-adapted.

FIG. 13A shows a cross sectional perspective view of a reagent tube 2200 having side wall 2201 and bottom 2202. Interior surface 2204 of the bottom is visible. A star-shaped cutout 2203 is shown in part, as three apical grooves.

Typically the star-shaped pattern is present as a raised portion on the lower interior surface of the tube. Thus, during manufacture of a reagent tube, such as by injection moulding, an outer portion of the mould is a cavity defining the exterior shape of the tube. An interior shape of the tube is formed by a mould positioned concentrically with the outer portion mould, and having a star-shaped structure milled out of its tip. Thus, when liquid plastic is injected into

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the space between the two portions of the mould, the star-shaped is formed as a raised portion on the bottom interior surface of the tube.

The exemplary star pattern 2203 shown in FIG. 13B in plan view resembles a “ship’s wheel” and comprises a center 2209, a circular ring 2207 centered on center 2209, and 8 radial segments configured as radial grooves 2205. Each groove meets the other grooves at center 2209, and has a radial end, also referred to as an apex or vertex. Star pattern 2203 has 8 grooves, such as 3, 4, 6, 10, or 12, would be consistent with the design herein. The number of grooves of the star should be minimum consistent with effective liquid pipetting and also spaced apart enough not to trap the tip of any of the pipette tips to be used in the liquid handling applications.

Center 2209 is typically positioned coincidentally with the geometric center of the bottom of reagent tube 2200. The tube is typically circular in cross-section, so identifying its center (e.g., as a crossing point of two diameters) is normally straightforward. Center 2209 may be larger than shown in FIG. 13B, such as may be a circular cutout or raised portion that exceeds in diameter of the region formed by the meeting point of grooves 2205.

Ring 2207 is an optional feature of star-shaped pattern 2203. Typically ring 2207 is centered about center 2209, and typically it also has a dimension that corresponds to the lower surface of a pipette tip. Thus, when a pipette tip “bottoms out” in the bottom of reagent tube 2200, the bottom of the pipette tip rests in contact with ring 2207. Ring 2207 is thus preferably a cut-out or recessed feature that can accommodate the pipette tip and assist in guiding its positioning centrally at the bottom of the tube. In other embodiments more than one, such as 2, 3, or 4 concentric rings 2207 are present.

The star pattern is configured to have dimensions that give an optimal flow-rate of liquid out of the reagent tube into a suitably positioned pipette tip. The star pattern is shown in FIG. 13B as being significantly smaller in diameter than the diameter of the tube at its widest point. The star pattern may have, in various embodiments, a diameter (measured from center 2209 at apex of a groove 2208) from 5-20% of the diameter of the reagent tube, or from 10-25% of the diameter of the reagent tube, or from 15-30% of the diameter of the reagent tube, or from 20-40% of the diameter of the reagent tube, or from 25-50% of the diameter of the reagent tube, or from 30-50% the diameter of the reagent tube, or from 40-60% the distance of the reagent tube, or from 50-75% the diameter of the reagent tube, or from 65-90% the diameter of the reagent tube.

The grooves 2205 are thus separated by ridges (occupying the space in between adjacent grooves). In the embodiment shown, the grooves are narrower (occupy a smaller radial angle) than the gaps between them. In other embodiments, the grooves may be proportionately wider than the gaps between them. In such embodiments, it may be more appropriate to describe them as having ridges instead of grooves. In other embodiments, the grooves and ridges that separate them are of equal widths at each radial distance from the center.

The grooves that form the apices of the star may be rounded in their lower surfaces, such as semi-circular in cross section, but are typically V-shaped. They may also be trapezoid in cross-section, such as having a wider upper portion than the bottom, which is flat, the upper portion and the bottom being connected by sloping walls.

In some embodiments, for ease of manufacture, the grooves end on the same level in the bottom of the tube.

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Thus the radial ends are all disposed on the circumference of a circle. In other embodiments, the grooves do not all end on the same level. For example, grooves may alternately end on different levels, and thus the ends are alternately disposed on the respective circumferences of two circles that occupy different planes in space from one another.

Grooves **2205** are shown in FIG. **13B** as having equal lengths (as measured from center **2209** to apex). This need not be so. In alternative embodiments, grooves may have different lengths from one another, for example, as alternating lengths on alternating grooves, where there are an even number of grooves. Furthermore, apices may be rounded, rather than pointed.

Typically the grooves taper uniformly in width and depth from center **2209** to each respective apex. Still other configurations are possible, such as a groove that follows a constant width, or depth, out to a particular radial extent, such as 30-60% of its length, and then narrows or becomes shallower towards its apex. Alternatively, a groove may start narrow at center **2209**, widen to a widest region near its midpoint of length, and then narrow towards its apex. Still other possibilities, not described herein, are consistent with the stellated pattern.

In a 0.3 ml tube, the width of each groove **2205** at its widest point is typically around 50 microns, and the width typically tapers uniformly from a widest point, closest to or at center **2209**, to the apex.

In a 0.3 ml tube, the depth of a groove at the deepest point is typically around 25-50 microns and the depth typically tapers uniformly from a deepest point, closed to or at center **2209**, at an apex.

In a 0.3 ml tube, the radius of the star formed from the grooves, measured as the shortest distance from center **2209** to apex, is typically around 0.5 mm, but may be from 0.1-1 mm, or from 0.3-2 mm.

In another embodiment, in a 0.3 ml tube, the grooves should be rounded off and less than 100 microns deep, or less than 50 microns deep, or less than 25 microns deep.

The stellated pattern typically has a rotation axis of symmetry, the axis disposed perpendicular to the bottom of the tube and through center **2209**, so that the grooves are disposed symmetrically about the rotation axis. By this is meant that, for n grooves, a rotation of $2\pi/n$ about the central (rotational) axis can bring each groove into coincidence with the groove adjacent to it.

The stellated shape shown in FIG. **13B** is not limiting in that it comprises a number of radially disposed grooves **2205**, and an optional circular ring **2207**. Other star-shaped geometries may be used, and, depending upon ease of manufacture, may be preferred. For example, a star can be created simply by superimposing two or more polygons having a common center, but offset rotationally with respect to one another about the central axis. (See, for example "star polygons" described at the Internet site mathworld.wplfram.com/StarPolygon.html.) Such alternative manners of creating star-shaped patterns are utilizable herein.

Liquid Dispenser

In various embodiments, preparation of a PCR-ready sample for use in subsequent diagnosis using the apparatus as further described herein, can include one or more of the following steps: contacting a neutralized polynucleotide sample with a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides (in some embodiments, the PCR reagent mixture can further include a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid); in some embodiments, the PCR reagent mixture can be in the form

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of one or more lyophilized pellets, as stored in a receptacle on a holder, and the method can further include reconstituting the PCR pellet with liquid to create a PCR reagent mixture solution. Various, such as one or more, of the liquid transfer operations associated with the foregoing steps can be accomplished by an automated pipette head.

A suitable liquid dispenser for use with the apparatus herein comprises one or more sensors; a manifold; one or more pumps in fluid communication with the manifold; one or more dispense heads in fluid communication with the manifold; and electrical connections that accept electrical signals from an external controller, wherein the liquid dispenser has no inlet or outlet for fluids, other than through the one or more pumps.

A cross-sectional view of an exemplary liquid dispenser is shown in FIG. **18**. The liquid dispenser is configured to carry out fluid transfer operations on two or more holders simultaneously. As shown in FIG. **18**, liquid dispenser **2105** can be mounted on a gantry having three degrees of translational freedom. Further embodiments can comprise a gantry having fewer than three degrees of translational freedom. The manner of mounting can be by a mechanical fastening such as one or more screws, as shown on the left hand side of FIG. **18**. A suitable gantry comprises three axes of belt-driven slides actuated by encoded stepper motors. The gantry slides can be mounted on a framework of structural angle aluminum or other equivalent material, particularly a metal or metal alloy. Slides aligned in x- and y-directions (directed out of and in the plane of FIG. **18** respectively) facilitate motion of the gantry across an array of holders, and in a direction along a given holder, respectively.

The z-axis of the gantry can be associated with a variable force sensor which can be configured to control the extent of vertical motion of the head during tip pick-up and fluid dispensing operations. Shown in FIG. **18**, for example, a pipette head **1803** can be mounted such that a force acting upwardly against the head can be sensed through a relative motion between the head and a force sensor. For example, when pipette head **1803** forces against a disposable pipette in the rack below it, an upward force is transmitted causing head **1803** to torque around pivot point **2102**, causing set screw **2104** to press against a force sensor. In turn, the force sensor is in communication with a processor or controller that controls at least the vertical motion of the liquid dispenser so that, thereby, the processor or controller can send instructions to arrest the vertical motion of the liquid dispenser upon receiving an appropriate signal from the force sensor. An exemplary force sensor suitable for use herein is available from Honeywell; its specification is shown in an appendix hereto. The force sensor mechanism shown in FIG. **18** is exemplary and one of many possible mechanisms capable of commanding the head during up pick-up and fluid dispensing operations. For example, as an alternative to a force sensor, a stall sensor that senses interruption in vertical motion of the one or more dispense heads upon contact with a sample tube or reagent holder may be used. Accordingly, as would be understood by one of ordinary skill in the art, the liquid dispenser as described herein is not limited to the specific mechanism shown in FIG. **18**.

The liquid dispenser further comprises a number of individually sprung heads **1803**, wherein each head is configured to accept a pipette tip from the one or more pipette tips in a holder. The liquid dispenser can be further configured such that no two heads accept pipette tips from the same holder. FIGS. **19A-C**, for example, depicts four individually sprung heads **1803**, but it is to be understood that the

dispenser is not limited to this number. For example, other number include 2, 3, 5, 6, 8, 10, or 12. Furthermore, the individually sprung heads **1803** are shown arranged in parallel to one another, but may be configured in other arrangements.

The liquid dispenser can further comprise computer-controlled pump **2100** connected to distribution manifold **1802** with related computer controlled valving. Distribution manifold **1802** can comprise a number of valves, such as solenoid valves **1801** configured to control the flow of air through the pipette tips in an exemplary embodiment, there are two valves for each pipette, and one additional valve to vent the pump. Thus, for a liquid dispenser having four pipette heads, there are nine valves. In another embodiment there is only one valve for each pipette, and one additional valve to vent the pump. However, the distribution manifold is not limited to comprising exactly nine solenoid valves.

The liquid dispenser is further configured to aspirate or dispense fluid in connection with analysis or preparation of solutions of two or more samples. The liquid dispense is also configured to dispense liquid into a microfluidic cartridge. Additionally, the liquid dispenser is configured to accept or dispense, in a single operation, an amount of 1.0 ml of fluid or less, such as an amount of fluid in the range 10 nl-1 ml.

The liquid dispenser is configured such that pump **2100** pumps air in and out of the distribution manifold. The distribution manifold comprises a microfluidic network that distributes air evenly amongst the one or more valves. Thus, by controlling flow of air through the manifold and various valves, pressure above the pipette heads can be varied so that liquid is drawn up into or expelled from a pipette tip attached to the respective pipette heads. In this way it is not necessary to supply compressed air via an air hose to the liquid dispenser. Neither is it necessary to provide liquid lines to the dispense head. Furthermore, no liquid reagents or liquid samples from the holders enters any part of the liquid dispenser, including the manifold. This aspect reduced complications from introducing air bubbles into samples or liquid reagents. An exemplary configuration of a distribution manifold is shown in FIG. 20.

As shown in the various figures, the entire liquid dispenser that moves up and down the z-axis is a self-contained unit having only electrical connections to a processor or controller, and mechanical connections to the gantry. The translational motions in three dimensions of the liquid dispenser can be controlled by a microprocessor, such as processor **980**. No fluid handling lines are associated with the dispenser. This design enables simplification of assembly of the instrument, minimizes contamination of the instrument and cross-contamination of samples between different instances of operation of the apparatus, increases efficiency of pumping (minimal dead volume) and enable easy maintenance and repair of the device. This arrangement also enable easy upgrading of features in the dispensing device, such as individual and independent pump control for each dispenser, individual pipette attachment or removal, ability to control the pitch of the pipettes, etc.

Another aspect of the apparatus relates to a sample identification verifier configured to check the identity of each of the number of nucleic-acid containing samples. Such sample identification verifiers can be optical character readers, bard code readers, or radio frequency tag readers, or other suitable readers, as available to one of ordinary skill in the art. A sample identification verifier can be mounted on the gantry, or attached to the liquid dispenser so that it moves in concert with the liquid dispenser. Alternatively, the sample identification verifier can be separately mounted and

can move independently of the liquid dispenser. In FIGS. **21** and **22**, for example, sample identification verifier **1701** is a bar-code reader attached to the liquid dispenser. The field of view of barcode scanner **1701** is non-linear, enabling it to detect light reflected by mirror **2300** from the barcoded clinical sample tube **2301** in disposable rack **2302**. The barcode scanner reads the barcode on the clinical sample tube thus identifying the presence and specifics of the sample tube. Because of use of a mirror, the scanner is configured either to read a bar-code printed in mirror image form (that is thus reflected into normal form), or to read a mirror image of a normal bar-code and to convert the mirror image to unreflected form via a computer algorithm.

Sample identification verifier is configured to communicate details of labels that it has detected to a processor or controller in the apparatus, thereby permitting sample identifying information to be associated with diagnostic results and other information relating to sample preparation, and extraction and amplification of nucleic acid therein.

In FIG. **23**, the sample identification verifier is positioned to read indicia from a microfluidic cartridge.

In certain embodiments, the liquid dispenser can also comprise one or more sensors **2001** (e.g., infra-red sensors) each of which detects the presence of a pipette tip in a rack. In FIG. **24**, for example, an infra-red sensor **2001** can have an infra-red emitter placed opposed to it, and the presence of disposable pipette tip **2000** obstructs the line of sight between the emitter and the detector, thus enabling determination of the presence or absence of the pipette tip. The disposal pipettes are configured perpendicular to pipette stripper-alignment plate **2003** as further described herein.

The liquid dispenser can also operate in conjunction with a motorized plate configured to strip the pipettes and align the pipettes during dispensing of fluid into a microfluidic cartridge, as further described herein.

FIGS. **25A** and **25B** show an exemplary device for stripping pipette tips from a liquid dispenser as further described herein. The pipette tips are aligned, all at the same pitch, above respective sockets (over a pipette tip sheath) in a holder. A metal plate having elongated holes lies over the sockets. The pipette tips are inserted part way down into the sheath through the elongated holes, and the metal plate is moved along in such a manner that the pipette tips are clamped by the elongated portion of the holes. When the liquid dispenser is moved up, the pipette tips become detached from their respective heads. When the metal plate is subsequently moved back to its initial position, the pipette tips remain in place in their respective sockets.

Heater Assembly & Magnetic Separator

A cross-sectional view of a heater unit of an exemplary heater assembly **1401** is shown in FIG. **18** (right hand panel). The heater assembly comprises one or more independently controllable heater units, each of which comprises a heat block. In certain embodiments there are 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 25, 30, 32, 36, 40, 48, or 50 heater units in a heater assembly. Still other numbers of heater units, such as any number between 6 and 100 are consistent with the description herein. The one or more heat blocks may be fashioned from a single piece of metal or other material, or may be made separately from one another and mounted independently of one another or connected to one another in some way. Thus, the term heater assembly connotes a collection of heater units but does not require the heater units or their respective heat blocks to be attached directly or indirectly to one another. The heater assembly can be configured so that each heater unit independently heats each of the one or more process tubes **1402**, for example by

permitting each of the one or more heat blocks to be independently controllable, as further described herein. In the configuration of FIG. 26, the heater assembly comprises one or more heat blocks **1403** each of which is configured to align with and to deliver heat to a process tube **1402**. Each heat block **1403** can be optionally secured and connected to the rest of the apparatus using a strip **1408** and one or more screws **1407** or other adhesive device. This securing mechanism is not limited to such as configuration.

Although a cross-sectional view of one heat block **1403** is shown in FIG. 26, it should be understood that this is consistent with having multiple heat blocks aligned in parallel to one another and such that their geometric mid-points all lie on a single linear axis, though it is not so limited in configuration. Thus, the one or more heat blocks may be positioned at different heights from one another, in groups or, alternately, individually, or may be staggered with respect to one another from left to right in FIG. 26 (right hand panel), in groups or alternately, or individually. Additionally, and in other embodiments, the heat blocks are not aligned parallel to one another but are disposed at angles relative to one another, the angles being other than 180°. Furthermore, although the heat block shown in FIG. 26 may be one of several that are identical in size, it is consistent with the technology herein that one or more heat blocks may be configured to accept and to heat process tubes of different sizes.

The exemplary heat block **1403** in FIG. 26 (right hand pane) is configured to have an internal cavity that partially surrounds a lower portion of process tube **1402**. In the heat block of FIG. 26, the internal cavity surrounds the lower portion of process tube **1402** on two sides but not the front side (facing away from magnet **1404**) and not the rear side (adjacent to magnet **1404**). In other embodiments, heat block **1403** is configured to surround the bottom of process tube **1402** on three sides, including the front side. Still other configurations of heat block **1403** are possible, consistent with the goals of achieving rapid and uniform heating of the contents of process tube **1402**. In certain embodiments, the heat block is shaped to conform closely to the shape of process tube **1402** so as to increase the surface area of the heat block that is in contact with the process tube during heating of the process tube. Thus, although exemplary heat block **1403** is shown having a conical, curve-bottomed cavity in which a complementary process tube is seated, other embodiments of the heat block **1403** have, for example, a cylindrical cavity with a flat bottom. Still other embodiments of heat block **1403** may have a rectilinear internal cavity such as would accommodate a cuvette.

Moreover, although heat block **1403** is shown as an L-shape in FIG. 26, which aids in the transmittal of heat from heating element **1501** and in securing the one or more heat blocks to the rest of the apparatus, it need not be so, as further described herein. For example, in some embodiments heating element **1501** may be positioned directly underneath process tube **1402**.

Each heat block **1403** is configured to have a low thermal mass while still maintaining high structural integrity and allowing a magnet to slide past the heat blocks and the process tubes with ease. A low thermal mass is advantageous because it allows heat to be delivered or dissipated rapidly, thus increasing the heating and cooling efficiency of the apparatus in which the heater assembly is situated. Factors that contribute to a low thermal mass include the material from which a heat block is made, and the shape that it adopts. The heat blocks **1403** can therefore be made of such

materials as aluminum, silver, gold, and copper, and alloys thereof, but are not so limited.

In one embodiment, the heat block **1403** has a mass of ~10 grams and is configured to heat up liquid samples having volumes between 1.2 ml and 10 μ l. Heating from room temperature to 65° C. for a 1 ml biological sample can be achieved in less than 3 minutes, and 10 μ l of an aqueous liquid such as a release buffer up to 85° C. (from 50° C.) in less than 2 minutes. The heat block **1403** can cool down to 50° C. from 85° C. in less than 3 minutes. The heat block **1403** can be configured to have a temperature uniformity of 65 \pm 4° C. for heating up 1 ml of sample and 85 \pm 3° C. for heating up 10 μ l of release buffer. These ranges are typical, but the heat block can be suitably scaled to heat other volumes of liquid at rates that are slower and faster than those described. This aspect of the technology is one aspect that contributes to achieving rapid nucleic acid extraction of multipole samples by combination of liquid processing steps, rapid heating for lysis, DNA capture and release and magnetic separation, as further described herein.

Not shown in FIG. 26, the heater assembly **1401** can also optionally be contained in an enclosure that surrounds the heat blocks **1403**. The enclosure can be configured to enable sufficient air flow around the process tubes and so as not to significantly inhibit rate of cooling. The enclosure can have a gap between it and the heat blocks to facilitate cooling. The enclosure can be made of plastic, but is not so limited. The enclosure is typically configured to appear aesthetic to a user.

As shown in FIG. 26, the heater assembly **1401** can also comprise one or more heating elements (e.g., a power resistor) **1501** each of which is configured to thermally interface to a heat block **1403** and dissipate heat to it. For example, in one embodiment, a power resistor can dissipate up to 25 Watts of power. A power resistor is advantageous because it is typically a low-cost alternative to a heating element. Other off-the-shelf electronic components such as power transistors may also be used to both sense temperature and heat. Although the heating element **1501** is shown placed at the bottom of the heat block **1403**, it would be understood that other configurations are consistent with the assembly described herein: for example, the heating element **1501** might be placed at the top or side of each heat block **1403**, or directly underneath process tube **1402**. In other embodiments, the heating element has other shapes and is not rectangular in cross section but may be curved, such as spherical or ellipsoidal. Additionally, the heating element may be moulded or shaped so that it conforms closely or approximately to the shape of the bottom of the process tube. Not shown in FIG. 26, the heater assembly can also comprise an interface material (e.g., Berquist q-pad, or thermal grease) between the heating element **1501** and the heat block **1403** to enable good thermal contact between the element and the heat block.

In the embodiment shown in FIG. 26, the heater assembly further comprises one or more temperature sensors **1502**, such as resistive temperature detectors, to sense the respective temperature of each heat block **1403**. Although a temperature sensor **1502** is shown placed at the bottom of the heat block **1403**, it would be understood that other configurations are consistent with the assembly described herein: for example, the temperature sensor might be placed at the top or side of each heat block **1403**, or closer to the bottom of process tube **1402** but not so close as to impede uniform heating thereof. As shown in the embodiment of FIG. 26, the heater assembly can further comprise an interface material (e.g., Berquist q-pad) **1503** configured to

enable good thermal contact between the sensor **1502** and the heat block **1403**, to thereby ensure an accurate reading.

Certain embodiments of the diagnostic or preparatory apparatus herein have more than one heater assembly as further described herein. For example, a single heater assembly may be configured to independently heat 6 or 12 process tubes, and an apparatus may be configured with two or four such heater assemblies.

The disclosure herein further comprises a magnetic separator, configured to separate magnetic particles, the separator comprising: one or more magnets affixed to a supporting member; a motorized mechanism configured to move the supporting member in such a manner that the one or more magnets move backwards and forwards along a fixed axis, and during at least a portion of the motion, the one or more magnets maintain close proximity to one or more receptacles which contain the magnetic particles in solution; and control circuitry to control the motorized mechanism.

The disclosure herein still further includes an integrated magnetic separator and heater, comprising: a heater assembly, wherein the heater assembly comprises a plurality of independently controllable heater units, each of which is configured to accept and to heat one of a plurality of process tubes; one or more magnets affixed to a supporting member; a motorized mechanism configured to move the supporting member in such a manner that the one or more magnets move backwards and forwards along a fixed axis, and during at least a portion of the motion the one or more magnets maintain close proximity to one or more of the process tubes in the heater assembly, wherein the one or more process tubes contain magnetic particles; and control circuitry to control the motorized mechanism and to control heating of the heater units.

Typically, each of the one or more receptacles is a process tube, such as for carrying out biological reactions. In some embodiments, close proximity can be defined as a magnet having a face less than 2 mm away from the exterior surface of a process tube without being in contact with the tube. It can still further be defined to be less than 1 mm away without being in contact with the tube, or between 1 and 2 mm away.

Typically the magnetic particles are microparticles, beads, or microspheres capable of binding one or more biomolecules, such as polynucleotides. Separating the particles, while in solution, typically comprises collecting and concentrating, or gathering, the particles into one location in the inside of the one or more receptacles.

An exemplary magnetic separator **1400** is shown in FIG. **27**, configured to operate in conjunction with heater assembly **1401**. The magnetic separator **1400** is configured to move one or more magnets relative to the one or more process tubes **1402**. While the magnet **1404** shown in FIG. **27** is shown as a rectangular block, it is not so limited in shape. Moreover, the configuration of FIG. **27** is consistent with either having a single magnet that extends across all heat blocks **1403** or having multiple magnets operating in concert and aligned to span a subset of the heat blocks, for example, aligned collinearly on the supporting member. The magnet **1404** can be made of neodymium (e.g., from K & J Magnetics, Inc.) and can have a magnetic strength of 5,000-15,000 Gauss (Brmax). The poles of the magnets **1404** can be arranged such that one pole faces the heat blocks **1403** and the other faces away from the heat blocks.

Further, in the embodiment shown in FIG. **27**, the magnet **1404** is mounted on a supporting member **1505** that can be raised up and down long a fixed axis using a motorized shaft **1405**. The fixed axis can be vertical. In the embodiment

shown in FIG. **27**, a geared arrangement **1406** enables the motor **1601** to be placed perpendicular to the shaft **1405**, thereby saving space in the apparatus in which magnetic separator **1400** is situated. In other embodiments, the motor is placed underneath shaft **1405**. It would be understood that other configurations are consistent with the movement of the magnet relative to the process tubes, including, but not limited to, moving the magnet from side-to-side, or bringing the magnet down from above. The motor can be computer controlled to run at a particular speed; for example at a rotational speed that leads to vertical motion of the magnet in the range 1-20 mm/s. The magnetic separator can thus be configured to move repetitively, e.g., up and down, from side to side, or backwards and forwards, along the same axis several times. In some embodiments there is more than one shaft that operates under motorized control. The presence of at least a second shaft has the effect of making the motion of the separator more smooth. In some embodiments, the supporting member rides on one more guiding members to ensure that the supporting member does not, for example, tip, twist, or yaw, or undergo other internal motions while moving (other than that of controlled motion along the axis) and thereby reduce efficacy of the separation.

The supporting member can also be configured to move the magnets between a first position, situated away from the one or more receptacles, and a second position situated in close proximity to the one or more receptacles, and is further configured to move at an amplitude about the second position where the amplitude is smaller than a distance between the first position and the second position as measured along the shaft.

Shown in FIGS. **26** and **27**, the heater assembly **1401** and the magnetic separator **1400** can be controlled by electronic circuitry such as on printed circuit board **1409**. The electronic circuitry **1409** can be configured to cause the heater assembly **1401** to apply heat independently to the process tubes **1402** to minimize the cost of heating and sensing. It can also be configured to cause the magnetic separator **1400** to move repetitively relative to the process tubes **1402**. The electronic circuitry **1409** can be integrated into a single printed circuit board (PCB). During assembly, a plastic guide piece can help maintain certain spacing between individual heat blocks **1403**. This design can benefit from use off-the-shelf electronics to control a custom arrangement of heat blocks **1403**.

Not shown in FIGS. **26** and **27**, an enclosure can cover the magnetic separator **1400** and the heater assembly **1401** for protection of sub-assemblies below and aesthetics. The enclosure can also be designed to keep the heat blocks **1403** spaced apart from one another to ensure efficiency of heating and cooling. The magnetic separator and heater assembly can, alternatively, be enclosed by separate enclosures. The one or more enclosures can be made of plastic.

Advantageously, the heater assembly and magnetic separator operate together to permit successive heating and separation operations to be performed on liquid materials in the one or more process tubes without transporting either the liquid materials or the process tubes to different locations to perform either heating or separation. Such operation is also advantageous because it means that the functions of heating and separation which, although independent of one another, are both utilized in sample preparation may be performed with a compact and efficient apparatus.

Cartridge Autoloader

An exemplary embodiment of a PCR amplification-detection system **2900** for use with a microfluidic cartridge is shown in FIG. **28**. The system **2900** performs and automates

the process of PCR on multiple nucleic-acid containing samples in parallel. The system **2900** comprises a depository **2907** for unused microfluidic cartridges, a cartridge auto-loader, a receiving bay for a microfluidic cartridge, a detector, and a waster tray **2903** configured to receive used microfluidic cartridges. In one embodiment, the cartridge autoloader comprises a cartridge pack **2901**, and a cartridge pusher **2904**.

The system **2900**, for illustration purposes, is configured so that a microfluidic cartridge moves in a plane and in a linear manner from the depository to the receiving bay, to the waste bin, but it need not be so arranged. For example, the waste cartridge bin **2903** can be aligned orthogonally, or any angle thereof, to the receiving bay, such as disposed behind it. Alternatively, each element (cartridge autoloader **2901**, receiving bay **2902**, and waste cartridge bin **2903**) can be configured in a step-wise manner where the cartridge pack **2901** is on the same, higher or lower level than the microfluidic PCR amplification-detection system **2902** and the microfluidic PCR amplification-detection system **2902** is on the same, higher or lower level than the waste cartridge bin **2903**. Another configuration could be that each of the three elements is not arranged linearly but at an angle to one another, although within the same plane.

FIG. **28** illustrates the cartridge pack **2901** and the waste cartridge bin **2903** below the plane of the receiving bay, and a detection system **2908** above the plane. This configuration is exemplary and it would be understood that these elements may be positioned above or below the plane in other embodiments.

FIG. **29** illustrates a depository for unused microfluidic cartridges. The depository can be configured to accept a number of individually stacked and individually loaded cartridges, or can be configured to accept a pack of cartridges. An exemplary cartridge pack has 24 cartridges. The depository may consist of a cage **2910** of any material that may or may not be transparent. For example it may be made of metal or plastic. The cartridge pack **2901** is not limited to twenty-four cartridges 106 per pack but may contain any number from 2 to 100. For example, other numbers such as 2, 4, 8, 10, 12, 16, 20, 30, 36, 40, 48, 50, or 64 are possible numbers of cartridges 106 per pack. Similarly, the depository may be configured to accept those numbers of cartridges, when individually stacked. In one embodiment, as in FIG. **29**, each cartridge **2906**, individually stacked, rests on ledges **2911** that protrude from the cage **2910**. However, other configurations are possible. For example, a cartridge **2906** may rest on recessed grooves made within the interior surfaces of cage **2910**. Furthermore, the cartridge pack **2901** may not need to be placed in a cage **2910**. The cartridge pack **2901** may itself include the necessary connections to bind securely to the apparatus to load the cartridges **2906**.

FIG. **30** is an illustration of an exemplary initial loading position of a cartridge pack **2901** in a depository when samples are loaded in the topmost cartridge in the pack. FIG. **30** shows the cartridge pack **2901** below a plane that contains a cartridge pusher. In other embodiments, the cartridge pack **2901** may be above the plane of a cartridge pusher where the pusher pushes the lowest cartridge out from the holder; or partly above and partly below in a holder **2920** where a cartridge pusher pushes a cartridge from the middle of the cartridge pack **2901**. In the embodiment shown, a topmost cartridge 106 is pushed along two guide rails **2905**. Alternatively, there may be more or fewer guide rails (such as one or three) or no guide rails at all so long as a cartridge **2906** can be caused to move to other required positions.

An exemplary cartridge pusher **2904** is shown in FIG. **31**. The cartridge pusher **2904** pushes a cartridge **2906** along guide rails **2905**, which allows a cartridge **2906** to travel to pre-calibrated positions by the mechanism of a stepper motor **2930**. However, it would be understood that the mechanism of transporting the cartridge **2906** is not limited to a stepper motor **2930** and thus other mechanisms are also consistent with the cartridge pusher **2904** as described herein.

FIG. **32** shows a used cartridge **2906** that has been pushed by the cartridge pusher **2904** into the waste cartridge bin **2903** after a PCR process has been completed. The embodiment shows a lipped handle **2940** that facilitates easy handling, such as emptying, of the bin **2903**. However, it would be understood that the handle **2940** is not limited to the style and shape shown.

An exemplary cartridge pack **2901**, before and after multiple PCR processes are completed are shown in FIG. **33**. After the cartridge pusher **2904** pushes a cartridge **2906** out of the cartridge pack **2901**, a spring **2950** at the bottom of the cartridge pack pushes against the lower surface of the stack of cartridges and causes the topmost cartridge to be made available for sample injection. The spring **2950** is not limited in number or type. Thus although a single helical or coiled spring is shown, it is consistent with the description herein that more than one helical or coiled springs could be used, such as 2, 3, or 4, and the alternatively a sprung metal strip, or several strips, could be used. Alternatively another mechanism for forcing the cartridges upwards could be deployed, such as pneumatic, hydraulic, or inflatable pressurized container, could be utilized.

It is to be noted that microfluidic cartridges, as further described herein, that have a raised lip along their edges to permit ease of stacking and/or storage in a pack or an auto-loader are particularly advantageous because the raised lips also introduce a stiffness into the cartridges and assist in keeping the fluid inlets on one cartridge away from those on another cartridge during storage and transport. The raised regions, which need not only be lip along each edge of a cartridge, also help minimize friction between the lower surface of one cartridge and the upper surface of another. Cartridge Receiving Bay

The present technology relates to an apparatus and related methods for amplifying, and carrying out diagnostic analyses on, nucleotides from biological samples. The apparatus is configured to act on a disposable microfluidic cartridge containing multiple sample lanes in parallel, and comprises a reusable instrument platform that can actuate on-cartridge operations, can detect and analyze the products of the PCR amplification in each of the lanes separately, in all simultaneously, or in groups simultaneously, and, optionally, can display the results on a graphical user interface.

FIG. **34** shows a perspective view of an exemplary cartridge **200** that contains multiple samples lanes, and exemplary read head **300** that contains detection apparatus for reading signals from cartridge **200**. Also shown in FIG. **34** is a tray **110** that, optionally, can accommodate cartridge **200** prior to insertion of the cartridge in a receiving bay. The apparatus described herein is able to carry out real-time PCR on a number of samples in cartridge **200** simultaneously. Preferably the number of samples is 12 samples, as illustrated with exemplary cartridge **200**, though other numbers of samples such as 4, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 are within the scope of the present description. In preferred operation of the apparatus, a PCR-ready solution containing the sample, and, optionally, one or more analyte-

specific reagents (ASR's) using other components of the apparatus, as further described herein, prior to introduction into cartridge 200.

In some embodiments, an apparatus includes a bay configured to selectively receive a microfluidic cartridge; at least one heat source thermally coupled to the bay; and coupled to a processor as further described herein, wherein the heat source is configured to heat individual sample lanes in the cartridge, and the processor is configured to control application of heat of the individual sample lanes, separately, in all simultaneously, or in groups simultaneously.

In some embodiments, an apparatus further includes at least one detector configured to detect a polynucleotide (nucleic acid) in a sample in one or more of the individual samples lanes, separately or simultaneously; wherein the processor is coupled to the detector to control the detector and to receive signals from the detector.

The bay can be a portion of the apparatus that is configured to selectively receive the microfluidic cartridge. For example, the bay and the microfluidic cartridge can be complementary in shape so that the microfluidic cartridge is selectively received in, e.g., a single orientation. For example, the microfluidic cartridge can have a registration member that fits into a complementary feature of the bay. The registration member can be, for example, a cut-out on an edge of the cartridge, such as a corner that is cut-off, or one or more notches that are made on one or more of the side. By selectively receiving the cartridge, the bay can help a user to place the cartridge so that the apparatus can properly operate on the cartridge. In this way, error-free alignment of cartridges can be achieved. Moreover, the cartridge can be designed to be slightly smaller than the receiving bay by approximately 200-300 micron for easy placement and removal of the cartridge. The apparatus can further include a sensor configured to sense whether the microfluidic cartridge is selectively received.

The bay can also be configured so that various components of the apparatus that can operate on the microfluidic cartridge (heat sources, detectors, force members, and the like) are positioned to properly operate on the microfluidic cartridge. For example, a contact heat source can be positioned in the bay such that it can be thermally coupled to a distinct location at a microfluidic cartridge that is selectively received in the receiving bay.

Alternatively, in connection with alignment of microheaters in the heater module with corresponding heat-requiring microcomponents (such as valves, pumps, gates, reaction chambers, etc), the microheaters can be designed to be slightly bigger than the heat requiring microfluidic components so that even though the cartridge may be off-centered from the heater, the individual components can still function effectively.

The detector 300 can be, for example, an optical detector, as further described herein. For example, the detector can include a light source that selectively emits light in an absorption band of a fluorescent dye, and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. Alternatively, for example, the optical detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye; or for example, the optical detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a

fluorescent polynucleotide probe or a fragment thereof; or for example, the optical detector can be configured to independently detect a plurality of fluorescent dyes at a plurality of different locations on a microfluidic cartridge, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof in a different sample.

The heat source can be, for example, a heat source such as a resistive heater or network of resistive heaters, a reversible heat source such as a liquid-filled heat transfer circuit or a thermoelectric element, a radiative heat source such as a xenon lamp, and the like.

In preferred embodiments, the at least one heat source can be a contact heat source selected from a resistive heater (or network thereof), a radiator, a fluidic heat exchanger and a Peltier device. The contact heat source can be configured at the receiving bay to be thermally coupled to one or more distinct locations of a microfluidic cartridge received in the bay, whereby the distinct locations are selectively heated. At least one additional contact heat source can be included, wherein the contact heat sources are each configured at the bay to be independently thermally coupled to a different distinct location in a microfluidic cartridge received in the bay, whereby the distinct locations are independently heated. The contact heat source can be configured to be in direct physical contact with a distinct location of a microfluidic cartridge received in the bay. In various embodiments, each contact source heater can be configured to heat a distinct location having an average diameter in 2 dimensions from about 1 millimeter (mm) to about 15 mm (typically about 1 mm to about 10 mm), or a distinct location having a surface area of between about 1 mm² about 225 mm² (typically between about 1 mm² and about 100 mm², or in some embodiments between about 5 mm² and about 50 mm²).

In various embodiments, at least one heat source can be a radiative heat source configured to direct heat to a distinct location of a microfluidic cartridge received in the receiving bay.

In various embodiments, the apparatus includes one or more force members that are configured to apply force to thermally couple that at least one heat source to at least a portion of the microfluidic cartridge received in the bay. The one or more force members can be configured to operate a mechanical member at the microfluidic cartridge. At least one force member can be manually operated. At least one force member can be mechanically coupled to a lid at the receiving bay, whereby operation of the lid operates the force member.

In various embodiments, the force applied by the one or more force members can result in an average pressure at an interface between a portion of the receiving bay and a portion of the microfluidic cartridge of about 1 psi. The application of force is important to ensure consistent thermal contact between the heater wafer and the PCR reactor and microvalves in the microfluidic cartridge.

In various embodiments, the apparatus can further include a lid at the receiving bay, the lid being operable to at least partially exclude ambient light from the bay. The lid can be, for example, a sliding lid. The lid can include the optical detector. A major face of the lid at the bay can vary from planarity by less than about 100 micrometers, for example, less than about 25 micrometers. The lid can be configured to be removable from the apparatus. The lid can include a latching member that ensures that the lid is securely closed before amplification reactions are applied to the samples in the cartridge.

FIG. 35 shows a schematic cross-sectional view of a part of an apparatus as described herein, showing input of sample into a cartridge 200 via a pipette tip 10 (such as a disposable pipette) attached to an automated dispensing head, and an inlet 202. Although not shown, there are as many inlets 202 as samples to be input into cartridge 200. Inlet 202 is preferably configured to receive a pipette or the bottom end of a PCR tube and thereby accept sample for analysis with minimum waste, and with minimum introduction of air. Cartridge 200 is disposed on top of and in contact with a heater substrate 400. Read head 300 is positioned above cartridge 200 and a cover for optics 310 restricts the amount of ambient light that can be detected by the read head.

In various embodiments, a system as described herein can include both a microfluidic cartridge and the diagnostic apparatus.

Microfluidic Cartridge

One aspect of the present technology relates to a microfluidic cartridge including a first, second, and third, layers that together define a plurality of microfluidic networks, each network having various components configured to carry out PCR on a sample having one or more polynucleotides whose presence is to be determined. The cartridge includes one or more sample lanes in parallel, wherein each lane is independently associated with a given sample for simultaneous processing, and each lane contains an independently configured microfluidic network. An exemplary cartridge having such a construction is shown in FIG. 36. Such a cartridge is simple to manufacture, and permits PCR in a concentrated reaction volume (~4 μ l) and enables rapid thermocycling, at ~20 seconds per cycle.

Although other layers may be found in cartridges having comparable performance and ease of manufacture, the cartridge herein includes embodiments having only three layers in their construction: a substrate having an upper side and an opposed lower side, wherein the substrate comprises a microfluidic network having a plurality of sample lanes; a laminate attached to the lower side to seal the components of the microfluidic network, and provide an effective thermal transfer layer between a dedicated heating element and components in the microfluidic network; and a label, attached to the upper side that also covers and seals holes that are used in the manufacturing process to load microfluidic components such as valves. Thus, embodiments herein include microfluidic cartridges consisting of three layers, a substrate, a laminate, and a label, though other, additional, features other than layers may be consistent with such characterizations. Embodiments herein further include microfluidic cartridges consisting essentially of three layers, a substrate, laminate, and a label, though other, additional, features other than layers may be consistent with such characterizations. Furthermore, embodiments herein still further include microfluidic cartridges comprising three layers, a substrate, a laminate, and a label.

A microfluidic network can include, in fluidic communication, one or more components selected from the group consisting of: gates, valves such as thermally actuated valves, channels, vents, and reaction chambers. Particular components of exemplary microfluidic networks are further described elsewhere herein. The cartridge typically processes the sample by increasing the concentration of a polynucleotide to be determined.

A sample lane is a set of elements, controllable independently of those in another sample lane, by which a sample can be accepted and analyzed, according to methods described herein. A lane comprises at least a sample inlet, and a microfluidic component, as further described herein in

connection with a microfluidic cartridge. In some embodiments, each microfluidic network additionally comprises an overflow reservoir to contain extra liquid dispensed into the cartridge.

In various embodiments, a lane can include a sample inlet port, a first thermally actuated valve, a second thermally actuated valve, a PCR reaction chamber, and channels connecting the inlet port to the PCR reaction chamber via the first valve, and channels connecting the PCR reaction chamber to an exit vent via the second valve. The sample inlet valve can be configured to accept a quantity of sample at a pressure differential compared to ambient pressure of between about 100 to 5000 Pa. It should be noted that the lower the loading pressure, the higher the fill time for a aliquot of reaction mix to fill the microfluidic network. Applying more pressure will reduce the fill time, but if the time for which the pressure is applied is not determined correctly, the sample could be blown out through the microfluidic cartridge (if an end hydrophobic vent is not present). Therefore the time for which the pressure is applied should be properly determined, such as by methods available to one of ordinary skill in the art, to prevent underfill or overflow. In general, the fill time is inversely proportional to the viscosity of the solution. For example, FIG. 37 shows a microfluidic cartridge containing twelve independent sample lanes capable of independent (simultaneous or successive) processing of samples.

The microfluidic network in each lane is typically configured to carry out PCR on a PCR-ready sample, such as one containing nucleic acid (DNA or RNA) extracted from a raw biological sample using other aspects of the apparatus as further described herein. A PCR-ready sample in thus typically a mixture comprising the PCR reagent(s) and the neutralized polynucleotide sample, suitable for subjecting to thermal cycling conditions that create PCR amplicons from the neutralized polynucleotide sample. For example, a PCR-ready sample can include a PCR reagent mixture comprising a polymerase enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid and a plurality of nucleotides, and at least one probe that is selective for a polynucleotide sequence.

Typically, the microfluidic network is configured so that the time required for a microdroplet of sample to pass from the inlet to the second valve is less than 50% of the time required for the sample to travel up to the exit vent. Typically, the microfluidic network is designed to have an increased flow resistance downstream of the two valves without increasing the total volume of the microfluidic network in comparison to the amount required to fill from the first valve to the end vent of the network.

FIG. 38A shows a perspective view of a portion of an exemplary microfluidic cartridge 200 according to the present technology. The cartridge may be referred to as a multi-lane PCR cartridge with dedicated pipette inlets 202. Shown in FIG. 38A are various representative components of cartridge 200. For example, sample inlet 202 is configured to accept a syringe, a pipette, or a PCR tube containing a PCR ready sample. More than one inlet 202 is shown, wherein one inlet operates in conjunction with a single lane. Various components of microfluidic circuitry in each lane are also visible. For example, microvalves 204, and 206, and vents 208, which, as further described herein, is a microfluidic channel that is long enough to permit PCR to occur in a sample. Above PCR reactor 210 is a window 212 that permits optical detection, such as detection of fluorescence

from a fluorescent substance, such as a fluorogenic hybridization probe, in PCR reactor **210** when a detector is situated above window **212**.

A multi-lane cartridge is configured to accept a number of samples, in particular embodiments 12 samples, wherein the samples include at least a first sample and a second sample, wherein the first sample and the second sample each contain one or more polynucleotides in a form suitable for amplification. The polynucleotides in question may be the same as, or different from one another, in different lanes of a cartridge. The multi-sample cartridge comprises at least a first microfluidic network and a second microfluidic network, adjacent to one another, wherein each of the first microfluidic network and the second microfluidic network is as elsewhere described herein, and wherein the first microfluidic network accepts the first sample, and wherein the second microfluidic network accepts the second sample.

The sample inlets of adjacent lanes are reasonably spaced apart from one another to prevent any contamination of one sample inlet from another sample when a user introduces a sample into any one cartridge. In some embodiments, the sample inlets are configured so as to prevent subsequent inadvertent introduction of sample into a given lane after a sample has already been introduced into that lane.

In some embodiments, the multi-sample cartridge has a size substantially the same as that of a 96-well plate as is customarily used in the art. Advantageously, then, the cartridge may be used with plate handlers used elsewhere in the art. Still more preferably, however, the multi-sample cartridge is designed so that a spacing between the centroids of sample inlets is 9 mm, which is an industry-recognized standard. This means that, in certain embodiments the center-to-center distance between inlet holes in the cartridge that accept samples from PCR tubes, as further described herein, is 9 mm. The inlet holes are manufactured frustoconical in shape with an appropriate conical angle so that industry-standard pipette tips (2 μ l, 20 μ l, 200 μ l, volumes, etc.) fit snugly, entering from the widest point of the inlet. Thus, in certain embodiments, an inlet comprises an inverted frustoconical structure of at least 1 mm height, and having a diameter at its widest point that accepts entry of a pipette tip, of from 1-5 mm. The apparatus herein may be adapted to suit other, later-arising, industry standards for pipette tips not otherwise described herein. Typically the volume of sample accepted via an inlet into a microfluidic network in a sample lane is from 1-20 μ l, and may be from 3-5 μ l. The inlet hole can be designed to fit a pipette tip snugly and to create a good seal around the pipette tip, within the cone of the inlet hole. However, the cone is designed such that the sealing is reversible because it is undesirable if the seal is so tight that the cartridge can be pulled away from its tray, or location in the receiving bay, when the pipette tips are lifted after the dispensing operations.

FIG. 37 shows a plan view of an exemplary microfluidic cartridge having 12 lanes. The inlet ports have a 6 mm spacing, so that, when used in conjunction with an automated sample loader having 4 heads, spaced equidistantly at 9 mm apart, the inlets can be loaded in three batches of 4 inlets: e.g., inlets 1, 4, 7, and 10 together, followed by 2, 5, 8, and 11, then finally 3, 6, 9, and 12, wherein the 12 inlets are numbered consecutively from one side of the cartridge to the other.

FIG. 39A shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIGS 38A and 38B. FIG. 38B shows another plan view (left panel) of another representative microfluidic circuit found in one lane of a multi-lane car-

tridge such as shown in FIG. 36, and shows how the circuit is visible through the cartridge construction (right panel). Other configurations of microfluidic network would be consistent with the function of the cartridges and apparatus described herein. In sequence, sample is introduced through liquid inlet **202**, and optionally flows into a bubble removal vent channel **208** (which permits adventitious air bubbles introduced into the sample during entry, to escape), and continues along a channel **216**. Typically, when using a robotic dispenser of liquid sample, the volume is dispensed accurately enough that formation of bubbles is not a significant problem, and the presence of vent channel **208** is not necessary.

Throughout the operation of cartridge **200** the fluid is manipulated as a microdroplet (not shown in FIGS. 39A,B). Valves **204** and **206** are shown in FIG. 39A as double-valves, having a source of thermally responsive material (also referred to as a temperature responsive substance) on either side of the channel where they are situated. However, valves **204** and **206** may either or both be single valves that have a source of thermally responsive material on only one side of the respective channels. Valves **204** and **206** are initially open, so that a microdroplet of sample-containing fluid can be pumped into PCR reactor **210** from inlet hole **202**. Upon initiating of processing, the detector present on top of the PCR reactor checks for the presence of liquid in the PCR reactor, and then closes valves **204** and **206** to isolate the PCR reaction mix from the channels on either side.

The PCR reactor **210** is a microfluidic channel that is heated through a series of cycles to carry out amplification of nucleotides in the sample, as further described herein. Typically the PCR reactor has a volume of 3-5 μ l, in particular, 4 μ l. The inside walls of the channel in the PCR reactor are made very smooth and polished to a shiny finish (for example, using a polish selected from SPI A1, SPI A2, SPI A3, SPI b1, or SPI B2) during manufacture. This is in order to minimize any microscopic air trapping in the surface of the PCR reactor, which would causing bubbling during the thermocycling steps. The presence of bubbles especially in the detection region of the PCR reactor might cause a false reading for the PCR reaction. Furthermore, the PCR reactor **210** is made shallow such that the temperature gradient across the depth of the channel is minimized. The region of the cartridge **212** above PCR reactor **210** permits a detector to monitor progress of the reaction and also to detect fluorescence from a probe that binds to a quantity of amplified nucleotide. The region **212** is made of thinner material than the rest of the cartridge so as to permit the PCR reactor to be more responsive to a heating cycle (for example, to rapidly heat and cool between temperatures appropriate for denaturing and annealing steps), and so as to reduce glare, autofluorescence, and undue absorption of fluorescence. Both valves **204** and **206** are closed prior to thermocycling to prevent any evaporation of liquid, bubble generation, or movement of fluid from the PCR reactor.

End vent **214** prevents a user from introducing any excess amount of liquid into the microfluidic cartridge, as well as playing a role of containing any sample from spilling over to unintended parts of the cartridge. A user may input sample volumes as small as an amount to fill from the bubble removal vent to the middle of the PCR reactor, or up to valve **204** or beyond valve **204**. The use of microvalves prevents both loss of liquid or vapor thereby enabling even a partially filled reactor to successfully complete a PCR thermocycling reaction. The application of pressure (such as \sim 1 psi) to contact the cartridge to the heater of the instrument assists in achieving better thermal contact between the heater and the

heat-receivable parts of the cartridge, and also prevents the bottom laminate structure from expanding, as would happen if the PCR channel was partially filled with liquid and the entrapped air would be thermally expanded during thermo-cycling.

In various embodiments, the microfluidic network can optionally include at least one hydrophobic vent additional to the end vent.

After PCR has been carried out on a sample, and presence or absence of a polynucleotide of interest has been determined, it is preferred that the amplified sample remains on the cartridge and that the cartridge is either used again (if one or more lanes remain open), or disposed of. Should a user wish to run a post amplification analysis, such as gel electrophoresis, the user may pierce a hole through the laminate of the cartridge, and recover an amount—typically about 1.5 microliter—of PCR products. The user may also place the individual PCR lane on a special narrow heated plate, maintained at a temperature to melt the wax in the valve, and the aspirate the reacted sample from the inlet hole of that PCR lane.

In various embodiments, the microfluidic network can optionally include at least one reservoir configured to contain waste.

In various embodiments, the microfluidic cartridge can further include a label, such as a computer-readable or scannable label. For example, the label can be a bar code, a radio frequency tag, or one or more computer-readable, or optionally scannable, characters. The label can be positioned such that it can be read by a sample identification verifier as further described herein.

In various embodiments, during transport and storage, the microfluidic cartridge can be further surrounded by a sealed pouch. The microfluidic cartridge can be sealed in the pouch with an inert gas. The microfluidic cartridge can be disposable.

Microfluidic cartridge **200** can be fabricated as desired. Typically, the microfluidic cartridge layer includes a layer of polypropylene or other plastic label with pressure sensitive adhesive (typically between about 50 and 150 microns thick) configured to seal the wax loading holes of the valves, tap air used for valve actuation, and serve as a location for operator markings. This layer can be in two separate pieces, though it would be understood by one of ordinary skill in the art that in many embodiments a single piece layer would be appropriate.

The microfluidic substrate layer, is typically injection molded out of a plastic, preferably a zeonor plastic (cyclic olefin polymer), having a PCR channel and valve channels on a first side, and vent channels and various inlet holes, including wax loading holes and liquid inlet holes, on a second side (disposed toward the label). Typically, all of the microfluidic networks together, including the PCR reactors, the inlet holes and the valves for isolating the PCR reaction chambers, are defined in a single substrate. The substrate is made of a material that confers rigidity on the substrate and cartridge, and is impervious to air or liquid, so that entry or exit of air or liquid during operation of the cartridge is only possible through the inlet or the vent.

Channels of a microfluidic network in a lane of cartridge **200** typically have at least one sub-millimeter cross-sectional dimension. For example, channels of such a network may have a width and/or a depth of about 1 mm or less (e.g., about 750 microns or less, about 500 microns, or less, about 250 microns or less).

The cartridge can further include a heat sealable laminate layer **222** (typically between about 100 and about 125

microns thick) attached to the bottom surface of the microfluidic substrate using, for example, heat bonding, pressure bonding, or a combination thereof. The laminate layer **222** may also be made from a material that has an adhesive coating on one side only, the side being the side that contacts the underside of the microfluidic substrate. This layer may be made from a single coated tape having a layer of Adhesive 420, made by 3M. Exemplary tapes include single-sided variants of double sided tapes having produce nos. 9783, 9795, and 9795B, and available from 3M. Other acceptable layers may include tapes based on micro-capsule based adhesives.

In use, cartridge **200** is typically thermally associated with an array of heat sources configured to operate the components (e.g., valves, gates, and processing region **210**) of the device. In some embodiments, the heat sources are operated by an operating system, which operates the device during use. The operating system includes a processor (e.g., a computer) configured to actuate the heat sources according to a desired protocol. Processors configured to operate microfluidic devices are described in, e.g., U.S. application Ser. No. 09/819,105, filed Mar. 28, 2001, which application is incorporated herein by reference.

Table 1 outlines volumes, pumping pressures, and operation times associated with various components of a microfluidic cartridge.

TABLE 1

Operation	Pumping Pressure	Displacement Volume	Time of Operation
Mixing displacements	~2 psi	10-25 μ l	1-2 minutes
Moving valve wax plugs	~1-2 psi	< 1 μ l	5-15 seconds

Operation	Pump Used	Pump Design	Pump Actuation
Mixing displacements	Expancel Pump	Same as above	Same as above
Moving valve wax plugs	Thermopneumatic pump	1 μ l of trapped air	Heat trapped air to ~70-90° C.

In some embodiments, a microfluidic cartridge further comprises a registration member that ensures that the cartridge is received by a complementary diagnostic apparatus in a single orientation, for example, in a receiving bay of the apparatus. The registration member may be a simple cut-out from an edge or a corner of the cartridge (as shown in FIG. **38A**), or may be a series of notches, or some other configuration of shapes that require a unique orientation of placement in the apparatus.

In some embodiments, the microfluidic cartridge comprises two or more positioning elements, or fiducials, for use when filling the valves with thermally responsive material. The positioning elements may be located on the substrate, typically the upper face thereof.

The microfluidic cartridge may also be stackable, such as for easy storage or transport, or may be configured to be received by a loading device, as further described herein, that holds a plurality of cartridges in close proximity to one another, but without being in contact. In order to accomplish either or both of these characteristics, the substrate may comprise two ridges, one of each situated along each of two opposite edges of the cartridge, the ridge disposed on the upper side of the substrate. Thus, where a cartridge has a rectangular aspect (ignoring any registration member or

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mechanical key), the two ridges may be situated along the long side, or along the short side, of the cartridge.

Valves

A valve is a microfluidic component that has a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). An exemplary double valve is shown in FIG. 40A. A double valve has two channels, one on either side of the channel whose flow it regulates, whereas a single valve has just one channel, disposed on one side of the channel whose flow it regulates.

Upon actuation, e.g., by application of heat, the valve transitions to a closed state that prevents material, such as a microdroplet of PCR-ready sample, from passing along the channel from one side of the valve to the other. For example, a valve includes one or more masses of a thermally responsive substance (TRS) that is relatively immobile at a first temperature and more mobile at a second temperature. A mass of TRS can be an essentially solid mass or an agglomeration of smaller particles that cooperate to obstruct the passage upon actuation. Examples of TRS's include a eutectic alloy (e.g., a solder), wax (e.g., an olefin), polymers, plastics, and combinations thereof. The first and second temperatures are insufficiently high to damage materials, such as polymer layers of a microfluidic cartridge in which the valve is situated. Generally, the second temperature is less than about 90° C. and the first temperature is less than the second temperature (e.g., about 70° C. or less).

For each mass associated with a valve, a chamber is in gaseous communication with the mass. Upon heating gas (e.g., air) in the chamber(s) and heating the one or more masses of TRS to the second temperature, gas pressure within a chamber moves the corresponding mass into the channel obstructing material from passing therealong. Other valves of the network have the same structure and operate in the same fashion as the valves described herein.

In order to make the valve sealing very robust and reliable, the flow channel at the valve junction is made narrow (150 µm wide and 150 µm deep or narrower) and the constricted channel is made at least 0.5 or 1 mm long such that the wax seals up a long narrow channel thereby reducing any leakage through the walls of the channel. In the case of a bad seal, there is leakage of fluid around the walls of the channel, past the wax. So the flow channel is narrowed as much as possible, and made longer, e.g., as long as ~1 mm. The valve operates by heating air in the wax-loading port, which forces the wax forwards in a manner so that it does not come back to its original position. In this way, both air and wax are heated during operation of the valve.

In various embodiments, the microfluidic network can include a bent valve as shown in FIG. 32B (as a single valve) to reduce the footprint of the valve on the cartridge and hence reduce cost per part for manufacturing highly dense microfluidic substrates. In the valve of FIG. 40B, the loading hole for TRS is in the center of the valve; the structures at either end are an inlet and an outlet and are shown for illustrative purposes only. Single valve shown.

In various embodiments, the network can include a curved valve as shown in FIG. 40C, also as a single valve, in order to reduce the effective cross-section of the micro-valve, enabling manufacture of cheaper dense microfluidic devices.

Vents

A hydrophobic vent (e.g., a vent in FIG. 41) is a structure that permits gas to exit a channel while limiting (e.g., preventing) liquid from exiting the channel. Typically,

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hydrophobic vents include a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane from Osmonics) that defines a wall of the channel. As discussed herein, hydrophobic vents can be used to position a microdroplet of sample at a desired location within a microfluidic network.

The hydrophobic vents of the cartridge are preferably constructed so that the amount of air that escapes through them is maximized while minimizing the volume of the channel below the vent surface. Accordingly, it is preferable that the vent is constructed so as to have a hydrophobic membrane of large surface area and a shallow cross section of the microchannel below the vent surface.

Bubble removal hydrophobic vents typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least about 7.5 mm) along a channel. The length of the hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydrophobic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less). Bubble vents are optional in the microfluidic networks of the microfluidic cartridges described herein.

The depth of the channel within the hydrophobic vent is typically about 75% or less (e.g., about 65% or less, about 60% or less) of than the depth of the channel upstream and downstream of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and downstream of the hydrophobic vent is about 250 microns.

A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50% wider) than a width of the channel upstream from the vent and downstream from the vent. For example, in an exemplary embodiment, the width of the channel within the hydrophobic vent is about 400 microns and the width of the channel upstream and downstream from the vent is about 250 microns.

Highly Multiplexed Embodiments

Embodiments of the apparatus and cartridge described herein may be constructed that have high-density microfluidic circuitry on a single cartridge that thereby permit processing of multiple samples in parallel, or in sequence, on a single cartridge. Preferred numbers of such multiple samples include 36, 40, 48, 50, 64, 72, 80, 96, and 100, but it would be understood that still other numbers are consistent with the apparatus and cartridge herein, where deemed convenient and practical.

Accordingly, different configurations of lanes, sample inlets, and associated heater networks are contemplated that can facilitate processing such numbers of samples on a single cartridge are within the scope of the instant disclosure. Similarly, alternative configurations of detectors for use in conjunction with such as highly multiplexed cartridge are also within the scope of the description herein.

In an exemplary embodiments, a highly multiplexed cartridge has 48 PCR channels, and has independent control of each valve in the channel, with 2 banks of thermocycling protocol per channel, as shown in FIG. 43. In the embodiment of FIG. 43, the heaters are arranged in three arrays. Heaters in two separate glass regions only apply heat to valves in the microfluidic networks in each lane. Because of the low thermal conductivity of glass, the individual valves may be heated separately from one another. This permits samples to be loaded into the cartridge at different times, and

passed to the PCR reaction chambers independently of one another. The PCR heaters are mounted on a silicon substrate—and are not readily heated individually, but thereby permit batch processing of PCR samples, where multiple samples from different lanes are amplified by the same set of heating/cooling cycles. It is preferably for the PCR heaters to be arranged in 2 banks (the heater arrays on the left and right are not in electrical communication with one another), thereby permitting a separate degree of sample control.

FIG. 42 shows a representative cartridge, revealing an inlet configuration for a 48-sample cartridge. The inlet configuration is compatible with an automatic pipetting machine that has dispensing heads situated at a 9 mm spacing. For example, such a machine having 4 heads can load 4 inlets at once, in 12 discrete steps, for the cartridge of FIG. 42.

FIG. 44 shows, in close, up an exemplary spacing of valves and lanes in adjacent lanes of a multi-sample microfluidic cartridge.

FIGS. 45 and 46 show close-ups of, respectively, heater arrays, and inlets, of the exemplary cartridge shown in FIG. 44.

FIGS. 47A-47C show various views of an embodiment of a radially-configured highly-multiplexed cartridge, having a number of inlets, microfluidic lanes, and PCR reaction zones.

The various embodiments shown in FIGS. 42-47C are compatible with liquid dispensers, receiving bays, and detectors that are configured differently from the specific examples described herein.

In another preferred embodiment (not shown in the FIGs.), a cartridge and apparatus is configured so that the read-head does not cover the sample inlets, thereby permitting loading of separate samples while other samples are undergoing PCR thermocycling.

Heater Configurations to Ensure Uniform Heating of a Region

Another aspect of the apparatus described herein relates to a method and apparatus for uniformly controlling the heating of a region of a microfluidic network that includes but is not limited to one or more microfluidic components. In an exemplary embodiment, multiple heaters can be configured to simultaneously and uniformly heat a region, such as the PCR reaction zone, of the microfluidic cartridge.

In preferred embodiments, a microfluidic cartridge having a microfluidic network comprising one or more microfluidic components is brought into contact with a heat source, within a suitably configured apparatus. The heat source is configured so that particular heating elements are situated to heat specific components of the microfluidic network of the cartridge.

FIG. 48 shows a cross-sectional view of an exemplary microfluidic cartridge to show relative location of PCR channel in relation to the heaters when the cartridge is placed in the instrument. The view in FIG. 48 is also referred to as a sectional-isometric view of the cartridge lying over the heater wafer. A window 903 above the PCR channel in the cartridge is shown in perspective view. PCR channel 901 (for example, 150 μ deep \times 700 μ wide), is shown in an upper layer of the cartridge. A laminate layer 905 of the cartridge (for example, 125 μ thick) is directly under the PCR channel 901. A further layer of thermal interface laminate 907 on the cartridge (for example, 125 μ thick) lies directly under the laminate layer 905. Heaters are situated in a further layer 913 directly under the thermal interface laminate. The heaters are photolithographically defined and etched metal layers of gold (typically about 3,000 Å thick). Layers of 400

Å of TiW are deposited on top and bottom of the gold layer to serve as an adhesion layer. The substrate used is glass, fused silica or quartz wafer having a thickness of 0.4 mm, 0.5 mm or 0.7 mm or 1 mm. A thin electrically-insulative layer of 2 μ m silicon oxide serves as an insulative layer on top of the metal layer. Additional thin electrically insulative layers such as a 2-4 μ m of Parylene may also be deposited on top of the Silicon oxide surface. Two long heaters 909 and 911, as further described herein, are also shown.

Referring to FIGS. 49A and 49B, the PCR reaction zone 1001, typically having a volume \sim 1.6 μ l, is configured with a long side and a short side, each with an associated heating element. The apparatus therefor preferably includes four heaters disposed along the sides of, and configured to heat, the PCR reaction zone, as shown in the exemplary embodiment of FIG. 38A: long top heater 1005, long bottom heater 1003, short left heater 1007, and short right heater 1009. The small gap between long top heater 1005 and long bottom heater 1003 results in a negligible temperature gradient (less than 1° C. across the width of the PCR channel at any point along the length of the PCR reaction zone) and therefore an effectively uniform temperature throughout the PCR reaction zone. The heaters on the short edges of the PCR reactor provide heat to counteract the gradient created by the two long heaters from the center of the reactor to the edge of the reactor. It would be understood by one of ordinary skill in the art that still other configurations of one or more heater(s) situated about a PCR reaction zone are consistent with the methods and apparatus described herein. For example, a 'long' side of the reaction zone can be configured to be heated by two or more heaters. Specific orientations and configurations of heaters are used to create uniform zones of heating even on substrates having poor thermal conductivity because the poor thermal conductivity of glass, or quartz, or fused silica substrates is utilized to help in the independent operation of various microfluidic components such as valves and independent operation of the various PCR lanes.

In preferred embodiments, each heater has an associated temperature sensor. In the embodiment of FIG. 49A, a single temperature sensor 1011 is used for both long heaters. A temperature sensor 1013 for short left heater, and a temperature sensor 1015 for short right heater are also shown. The temperature sensor in the middle of the reactor is used to provide feedback and control the amount of power supplied to the two long heaters, whereas each of the short heaters has a dedicated temperature sensor placed adjacent to it in order to control it. As further described herein, temperature sensors are preferably configured to transmit information about temperature in their vicinity to the processor at such times as the heaters are not receiving current that causes them to heat. This can be achieved with appropriate control of current cycles.

In order to reduce the number of sensor or heater elements required to control a PCR heater, we may use the heaters to sense as well as heat, and thereby obviate the need to have a separate dedicated sensor for each heater. In another embodiment, each of the four heaters may be designed to have an appropriate wattage, and connect the four heaters in series or in parallel to reduce the number of electronically-controllable elements from 4 to just 1, thereby reducing the burden on the electronics.

FIG. 49B shows expanded views of heaters and temperature sensors used in conjunction with a PCR reaction zone of FIG. 49A. Temperature sensors 1001 and 1013 are designed to have a room temperature resistance of approximately 200-300 ohms. This value of resistance is determined by controlling the thickness of the metal layer deposited

(e.g., a sandwich of 400 Å TiW/3000 Å Au/400 Å TiW), and etching the winding metal line to have a width of approximately 10-25 μm and 20-40 mm length. The use of metal in this layer gives it a temperature coefficient of resistivity of the order of 0.5-20° C./ohms, preferably in the range of 1.5-3° C./ohms. Measuring the resistance at higher temperatures will enable determination of the exact temperature of the location of these sensors.

The configuration for uniform heating, shown in FIG. 49A for a single PCR reaction zone, can be applied to a multi-lane PCR cartridge in which multiple independent PCR reactions occur.

Each heater can be independently controlled by a processor and/or control circuitry used in conjunction with the apparatus described herein. FIG. 50 shows thermal images, from the top surface of a microfluidic cartridge having heaters configured as in FIGS. 49A and 49B, when each heater in turn is activated, as follows: (A) Long Top only; (B) Long Bottom only; (C) Short Left only; (D) Short Right only; and (E) All Four Heaters on. Panel (F) show a view of the reaction zone and heaters on the same scale as the other image panels in FIG. 50. Also shown in the figure is a temperature bar.

Use of Cutaways in Cartridge Substrate to Improve Rate of Cooling During PCR Cycling

During a PCR amplification of a nucleotide sample, a number of thermal cycles are carried out. For improved efficiency, the cooling between each application of heat is preferably as rapid as possible. Improved rate of cooling can be achieved with various modifications to the heating substrate, as shown in FIGS. 51A-51C.

One way to achieve rapid cooling is to cutaway portions of the microfluidic cartridge substrate, as shown in FIG. 51A. The upper panel of FIG. 51A is a cross-section of an exemplary microfluidic cartridge taken along the dashed line A-A' as marked on the lower panel of FIG. 51A. PCR reaction zone 901, and representative heaters 1003 are shown. Also shown are two cutaway portions, one of which labeled 1201, that are situated alongside the heaters that are situated along the long side of the PCR reaction zone. Cutaway portions such as 1201 reduce the thermal mass of the cartridge, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction zone. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology.

Another way to achieve rapid cooling is to cutaway portions of the heater substrate, as shown in FIG. 51B. The lower panel of FIG. 51B is a cross-section of an exemplary microfluidic cartridge and heater substrate taken along the dashed line A-A' as marked on the upper panel of FIG. 51B, PCR reaction zone 901, and representative heaters 1003 are shown. Also shown are four cutaway portions, one of which labeled 1205, that are situated alongside the heaters that are situated along the long side of the PCR reaction zone. Cutaway portions such as 1205 reduce the thermal mass of the heater substrate, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction zone. Four separate cutaway portions are shown in FIG. 51B so that control circuitry to the various heaters is not disrupted. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology. These cutouts may be created by a method selected from: selective etching using wet etching processes, deep reactive ion etching, selective etching using

CO₂ laser or femtosecond laser (to prevent surface cracks or stress near the surface), selective mechanical drilling, selective ultrasonic drilling, or selective abrasive particle blasting. Care has to be taken to maintain mechanical integrity of the heater while reducing as much material as possible.

FIG. 51C shows a combination of cutouts and use of ambient air cooling to increase the cooling rate during the cooling stage of thermocycling. A substantial amount of cooling happens by convective loss from the bottom surface of the heater surface to ambient air. The driving force for this convective loss is the differential in temperatures between the glass surface and the air temperature. By decreasing the ambient air temperature by use of, for example, a peltier cooler, the rate of cooling can be increased. The convective heat loss may also be increased by keeping the air at a velocity higher than zero.

An example of thermal cycling performance obtained with a configuration as described herein, is shown in FIG. 52 for a protocol that is set to heat up to 92° C., and stay here for 1 second, then cool to 62° C., and stay for 10 seconds. Cycle time is about 29 seconds, with 8 seconds required to heat from 62° C. and stabilize at 92° C., and 10 seconds required to cool from 92° C., and stabilize at 62° C.

Manufacturing Process for Cartridge

FIG. 53 shows a flow-chart 2800 for an assembly process for an exemplary cartridge as further described herein. It would be understood by one of ordinary skill in the art, both that various steps may be performed in a different order from that set forth in FIG. 53, and additionally that any given step may be carried out by alternative methods to those set forth in the figure. It would also be understood that, where separate steps are illustrated for carrying out two or more functions, such functions may be performed synchronously and combined into single steps and be consistent with the overall process described herein.

At 2802, a laminate layer is applied to a microfluidic substrate that has previously been engineered to have a microfluidic network constructed in it; edges are trimmed from the laminate where they spill over the bounds of the substrate.

At 2804, wax is dispensed and loaded into the microvalves of the microfluidic network in the microfluidic substrate. An exemplary process for carrying this out is further described herein.

At 2806, the cartridge is inspected to ensure that wax from step 2804 is loaded properly and that the laminate from step 2802 adheres properly to the microfluidic substrate. If a substrate does not satisfy either or both of these tests, it is discarded. If substrates repeatedly fail either or both of these tests, then the wax dispensing, or laminate application steps, as applicable, are reviewed.

At 2808, a hydrophobic vent membrane is applied to, and heat bonded to, the top of the microfluidic substrate over the wax valves, and on the opposite face of the substrate from the laminate. Edges of the membrane that are in excess of the boundary of the substrate are trimmed.

At 2810, the assembly is inspected to ensure that the hydrophobic vent membrane is bonded well to the microfluidic substrate without heat-clogging the microfluidic channels. If any of the channels is blocked, or if the bond between the membrane and the substrate is imperfect, the assembly is discarded, and, in the case of repeated discard events, the foregoing process step is reviewed.

At 2812, a thermally conductive pad layer is applied to the bottom laminate of the cartridge.

At 2814, two label strips are applied to the top of the microfluidic substrate, one to cover the valves, and a second

to protect the vent membranes. It would be understood that a single label strip may be devised to fulfill both of these roles.

At 2816, additional labels are printed or applied to show identifying characteristics, such as a barcode #, lot # and expiry date on the cartridge. Preferably one or more of these labels has a space and a writable surface that permits a user to make an identifying annotation on the label, by hand.

At 2818, to facilitate transport and delivery to a customer, assembled and labeled cartridges are stacked and pack cartridges in groups, such as groups of 25, or groups of 10, or groups of 20, or groups of 50. Preferably the packaging is via an insert and/or moisture-free medium.
Exemplary Wax-Deposition Process

Deposition of wax in valves of the microfluidic network, as at step 2804 may be carried out with the exemplary equipment shown in FIGS. 54A and 54B. The DispenseJet Series DJ-9000 (FIGS. 54A and 54B) is a non-contact dispenser that provides high-speed delivery and exceptional volumetric control for various fluids, including surface mount adhesive, underfill, encapsulants, conformal coating, UV adhesives, and silver epoxy. The DJ-9000 jets in tight spaces as small as 200 micrometers and crates fillet wet-out widths as small as 300 micrometers on the dispensed side of a substrate such as a die. It dispenses fluid either as discrete dots or a rapid succession of dots to form a 100-micron (4 mil) diameter stream of fluid from the nozzle. It is fully compatible with other commercially available systems such as the Asymtek Century C-718/C-720, Millennium M-2000, and Axiom X-1000 Series Dispensing Systems.

A DJ-9000 is manufactured by Asymtek under manufacturing quality control standards aim to provide precise and reliable performance. Representative specifications of the apparatus are as follows.

Characteristic	Specification
Size	Width: 35 mm Height: 110 mm Depth: 100 mm
Weight	400 grams - dry
Feed Tube Assembly	Nylon - Fitting Polyurethane - Tube
Fluid Chamber	Type 303 Stainless Steel
Seat and Nozzle	300/400 Series S/S, Carbide
Needle Assembly	52100 Bearing Steel - Shaft Hard Chrome Plate Carbide - Tip
Fluid Seal	PEEK/Stainless Steel
Fluid Chamber O-Ring	Ethylene Propylene
Jet Body	6061-T6 Aluminum Nickel Plated
Needle Assembly Bearings	PEEK
Thermal Control Body	6061-T6 Aluminum Nickel Plated
Reservoir Holder	Acetyl
Reservoir Size	5, 10, or 30 cc (0.17, 0.34, or 1.0 oz)
Feed Tube Assembly Fitting	Femail Luer per ANSI/HIMA MD70.1-1983
Maximum Cycle Frequency	200 Hz.
Minimum Valve Air Pressure	5.5 bar (80psi)
Operating Noise Level	70 db*
Solenoid	24 VDC, 12.7 Watts
Thermal Control Heater	24 VDC, 14.7 Watts, 40 ohms
Thermal Control RTD	100 ohm, platinum
Maximum Heater Set Point	80° C.

*At Maximum Cycle Rate

An exploded view of this apparatus is shown in FIG. 54B. Theory of Operation of DJ-9000

The DJ-9000 has a normally closed, air-actuated, spring-return mechanism, which uses momentum transfer prin-

ciples to expel precise volumes of material. Pressurized air is regulated by a high-speed solenoid to retract a needle assembly from the seat. Fluid, fed into the fluid chamber, flows over the seat. When the air is exhausted, the needle travels rapidly to the closed position, displacing fluid through the seat and nozzle in the form of a droplet. Multiple droplets fired in succession can be used to form larger dispense volumes and lines when combined with the motion of a dispenser robot.

The equipment has various adjustable features: The following features affect performance of the DJ-9000 and are typically adjusted to fit specific process conditions.

Fluid Pressure should be set so that fluid fills to the set, but should not be influential in pushing the fluid through the seat and nozzle. In general, higher fluid pressure results in a larger volume of material jetted.

The Stroke Adjustment controls the travel distance of the Needle Assembly. The control is turned counterclockwise to increase needle assembly travel, or turned clockwise to decrease travel. An increase of travel distance will often result in a larger volume of material jetted.

The Solenoid Valve controls the valve operation. When energized, it allows air in the jet air chamber to compress a spring and thereby raise the Needle Assembly. When de-energized, the air is released and the spring forces the piston down so that the needle tip contacts the seat.

The seat and nozzle geometry are typically the main factors controlling dispensed material volume. The seat and nozzle size are determined based on the application and fluid properties. Other parameters are adjusted in accordance with seat and nozzle choices. Available seat and nozzle sizes are listed in the table hereinbelow.

Thermal Control Assembly: Fluid temperature often influences fluid viscosity and flow characteristics. The DJ-9000 is equipped with a Thermal Control Assembly that assures a constant fluid temperature.

Dot and Line Parameters: In addition to the DJ-9000 hardware configuration and settings, Dot and Line Parameters are set in a software program (referred to as FmNT) to control the size and quality of dots and line dispensed.
Wax Loading in Valves

FIGS. 55A and 55B show how a combination of controlled hot drop dispensing into a heated microchannel device of the right dimensions and geometry is used to accurately load wax into a microchannel of a microfluidic cartridge to form a valve. The heated dispenser head can be accurately position over an inlet hole of the microchannel in the microfluidic device, and can dispense molten wax drops in volumes as small as 75 nanoliters with an accuracy of 20%. The inlet hole of the microchannel device is dimensioned in such as way that the droplet of 75 nl can be accurately shot to the bottom of the inlet hole using, for example, compressed air, or in a manner similar to an inkjet printing method. The microchannel device is maintained at a temperature above the melting point of the wax thereby permitting the wax to stay in a molten state immediately after it is dispensed. After the drop falls to the bottom of the inlet hole, the molten wax is drawn into the narrow channel by capillary action. The volume of the narrow section is designed to be approximately equal to a maximum typical amount that is dispensed into the inlet hole.

Heater Multiplexing (Under Software Control)

Another aspect of the apparatus described herein, relates to a method for controlling the heat within the system and its components, as illustrated in FIG. 56. The method leads to a greater energy efficiency of the apparatus described herein,

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because not all heaters are heating at the same time, and a given heater is receiving current for only part of the time.

Generally, the hating of microfluidic components, such as a PCR reaction zone, is controlled by passing currents through suitably configured microfabricated heaters. The heating can be further controlled by periodically turning the current on and off with varying pulse width modulation (PWM), wherein pulse width modulation refers to the on-time/off-time ratio for the current. The current can be supplied by connecting a microfabricated heater to a high voltage source (for example, 30V), which can be gated by the PWM signal. In some embodiments, the device includes 48 PWM signal generators. Operation of a PWM generator includes generating a signal with a chose, programmable period (the end count) and granularity. For instance, the signal can be 4000 μ s (micro-seconds) with a granularity of 1 μ s, in which case the PWM generator can maintain a counter beginning at zero and advancing in increments of 1 μ s until it reaches 4000 μ s, when it returns to zero. Thus, the amount of heat produced can be adjusted by adjusting the end count. A high end count corresponds to a greater length of time during which the microfabricated heater receives current and therefore a greater amount of heat produced.

In various embodiments, the operation of a PWM generator can also include a programmable start count in addition to the aforementioned end count and granularity. In such embodiments, multiple PWM generators can produce signals that can be selectively non-overlapping (e.g., by multiplexing the on-time of the various heaters) such that the current capacity of the high voltage power is not exceeded. Multiple heaters can be controlled by different PWM signal generators with varying start and end counts. The heaters can be divided into banks, whereby a bank defines a group of heaters of the same start count. For example, 36 PWM generators can be grouped into six different banks, each corresponding to a certain portion of the PWM cycle (500 ms for this example). The end count for each PWM generator can be selectively programmed such that not more than six heaters will be on at any given time. A portion of a PWM cycle can be selected as dead time (count 3000 to 4000 for this example) during which no heating takes place and sensitive temperature sensing circuits can use this time to sense the temperature. The table below represents a PWM cycle for the foregoing example:

	Start Count	End Count	Max End count
Bank 1			
PWM generator#1	0	150	500
PWM generator#2	0	220	500
...
PWM generator#6	0	376	500
Bank 2			
PWM generator#7	500	704	1000
PWM generator#8	500	676	1000
...
PWM generator#12	500	780	1000
Bank 3			
PWM generator#13	1000	1240	1500
PWM generator#14	1000	1101	1500
...
PWM generator#18	1000	1409	1500

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-continued

	Start Count	End Count	Max End count
Bank 4			
PWM generator#13	1500	1679	2000
PWM generator#14	1500	1989	2000
...
PWM generator#18	1500	1502	2000
Bank 5			
PWM generator#25	2000	2090	2500
PWM generator#26	2000	2499	2500
...
PWM generator#30	2000	2301	2500
Bank 6			
PWM generator#31	2500	2569	3000
PWM generator#32	2500	2790	3000
...
PWM generator#36	2500	2679	3000

Use of Detection System to Measure/Detect Fluid in PCR Chamber

The apparatus optionally has a very sensitive fluorescence detector that is able to collect fluorescence light from the PCR chamber **210** of a microfluidic cartridge. This detector is used to detect the presence of liquid in the chamber, a measurement that determines whether or not to carry out a PCR cycle. A background reading is taken prior to filling the chamber with liquid. Another reading is taken after microfluidic operations have been performed that should result in filling the PCR chamber with liquid. The presence of liquid alters the fluorescence reading from the chamber. A programmable threshold value is used to tune an algorithm programmed into the processor (for example, the second reading has to exceed the first reading by 20%). If the two readings do not differ beyond the programmed margin, the liquid is deemed to not have entered the chamber, and a PCR cycle is not initiated for that chamber. Instead, a warning is issued to a user.

Computer Program Product

In various embodiments, a computer program product for use with the apparatus herein includes computer readable instructions thereon for operating the apparatus.

In various embodiments, the computer program product can include one or more instructions to cause the system to: output an indicator of the placement of the microfluidic cartridge in the bay; read a sample label on a microfluidic cartridge label; output directions for a user to input a sample identifier; output directions for a user to load a sample transfer member with the PCR-ready sample; output directions for a user to introduce the PCR-ready sample into the microfluidic cartridge; output directions for a user to place the microfluidic cartridge in the receiving bay; output directions for a user to close the lid to operate the force member; output directions for a user to pressurize the PCR-ready sample in the microfluidic cartridge by injecting the PCR-ready sample with a volume of air between about 0.5 mL and about 5 mL; and output status information for sample progress from one or more lanes of the cartridge.

In various embodiments, the computer program product can include one or more instructions to cause the system to: heat the PCR ready-sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide; contact the neutralized polynucleotide sample or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; independently contact each of the neutralized polynucleotide sample

and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide; contact the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; output a determination of the presence of a polynucleotide sequence in the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; and/or output a determination of a contaminated result if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof.

In various embodiments, the computer program product can include one or more instructions to cause the system to automatically conduct one or more of the steps of the method.

In various embodiments, the microfluidic cartridge comprises two or more sample lanes, each including a sample inlet valve, a bubble removal vent, a thermally actuated pump, a thermally actuated valve, and a PCR reaction zone, wherein the computer readable instructions are configured to independently operate one or more components of each said lane in the system, independently of one another, and for causing a detector to measure fluorescence from the PCR reaction zone.

Sample

In various embodiments, the sample can include a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides. The PCR reagent mixture can be in the form of one or more lyophilized pellets and the steps by which the PCR-ready sample is prepared can involve contacting the PCR pellet with liquid to create a PCR reagent mixture solution. In yet another embodiment, each of the PCR lanes may have dried down or lyophilized ASR reagents preloaded such that the user only need to input prepared polynucleotide sample into the PCR. In another embodiment, the PCR lanes may have only the application-specific probes and primers premeasured and preloaded, and the user inputs a sample mixed with the PCR reagents.

In various embodiments, the microfluidic network can be configured to couple heat from an external heat source to a sample mixture comprising PCR reagent and neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

In various embodiments, the PCR ready sample can further include a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid. In various embodiments, the PCR-ready sample further includes a sample buffer, and at least one probe that is selective for a polynucleotide sequence, e.g., the polynucleotide sequence that is characteristic of a pathogen selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the microfluidic cartridge can accommodate a negative control polynucleotide, wherein the microfluidic network can be configured to independently carry out PCR on each of a neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide. Each lane of a multi-lane cartridge as described herein can perform two reactions because of the

presence of two fluorescence detection systems per lane. A variety of combinations of reactions can be performed in the cartridge, such as two sample reactions in one lane, a positive control and a negative control in two other lanes; or a sample reaction and an internal control in one lane and a negative control in a separate lane.

In various embodiments, the sample can include at least one probe that can be selective for a polynucleotide sequence, wherein the steps by which the PCR-ready sample is prepared involve contacting the neutralized polynucleotide sample or a PCR amplicon thereof with the probe. The probe can be a fluorogenic hybridization probe. the fluorogenic hybridization probe can include a polynucleotide sequence coupled to a fluorescent reporter dye and a fluorescence quencher dye. The PCR reagent mixture can further include a positive control plasmid and a plasmid fluorogenic hybridization probe selective for at least a portion of the plasmid and the microfluidic cartridge can be configured to allow independent optical detection of the fluorogenic hybridization probe and the plasmid fluorogenic hybridization probe.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism, for example any organism that employs deoxyribonucleic acid or ribonucleic acid polynucleotides. Thus, the probe can be selective for any organism. Suitable organisms include mammals (including humans), birds, reptiles, amphibians, fish, domesticated animals, wild animals, extinct organisms, bacteria, fungi, viruses, plants, and the like. The probe can also be selective for components of organisms that employ their own polynucleotides, for example mitochondria. In some embodiments, the probe is selective for microorganisms, for example, organisms used in food production (for example, yeasts employed in fermented products, molds or bacteria employed in cheeses, and the like) or pathogens (e.g., of humans, domesticated or wild mammals, domesticated or wild birds, and the like). In some embodiments, the probe is selective for organisms selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of *Staphylococcus* spp., e.g., *S. epidermidis*, *S. aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus*; *Streptococcus* (e.g., α , β or γ -hemolytic, Group A, B, C, D or G) such as *S. pyogenes*, *S. agalactiae*; *E. faecalis*, *E. durans*, and *E. faecium* (formerly *S. faecalis*, *S. durans*, *S. faecium*); nonenterococcal group D streptococci, e.g., *S. bovis* and *S. equines*; *Streptococci viridans*, e.g., *S. mutans*, *S. sanguis*, *S. salivarius*, *S. mitior*, *A. milleri*, *S. constellatus*, *S. intermedius*, and *S. anginosus*; *S. iniae*; *S. pneumoniae*; *Neisseria*, e.g., *N. meningitidis*, *N. gonorrhoeae*, saprophytic *Neisseria* sp; *Erysipelothrix*, e.g., *E. rhusiopathiae*; *Listeria* spp., e.g., *L. monocytogenes*, rarely *L. ivanovii* and *L. seeligeri*; *Bacillus*, e.g., *B. Anthracis*, *B. cereus*, *B. subtilis*, *B. subtilis niger*, *b. thuringiensis*; *Nocardia asteroides*; *Legionella*, e.g., *L. pneumonophila*, *Pneumocystis*, e.g., *P. Carinii*; *Enterobacteriaceae* such as *Salmonella*, *Shigella*, *Escherichia* (e.g., *E. coli*, *E. coli* O157:H7); *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Morganella*, *Providencia*, *Yersinia*, and the like, e.g., *Salmonella*, e.g., *S. typhi*, *S. paratyphi* A, B (*S. schottmuelleri*), and C (*S. hirschfeldii*), *S. dublin*, *S. choleraesuis*, *S. enteritidis*, *S. typhimurium*, *S. heidelberg*, *S. newport*, *S. infantis*, *S. agona*, *S. momevideo*, and *S. saint-paul*; *Shigella* e.g., subgroups: A, B, C, and D, such as *S. flexneri*, *S. sonnei*, *S. boydii*, *S. dysenteriae*;

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Proteus (*P. mirabilis*, *P. vulgaris*, and *P. myxofaciens*), *Morganella* (*M. morganii*); *Providencia* (*P. rettgeri*, *P. alcalifaciens*, and *P. stuartii*); *Yersinia*, e.g., *Y. pestis*, *Y. enterocolitica*; *Haemophilus*, e.g., *H. influenzae*, *H. parainfluenzae*, *H. aegyptius*, *H. ducreyi*; *Brucella*, e.g., *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*; *Francisella*, e.g., *F. tularensis*; *Pseudomonas*, e.g., *P. aeruginosa*, *P. paucimobilis*, *P. putida*, *P. fluorescens*, *P. acidovorans*, *Burkholderia* (*Pseudomonas*) *pseudomallei*, *Burkholderia* *mallii*, *Burkholderia* *cepacia* and *Stenotrophomonas maltophilia*; *Campylobacter*, e.g., *C. fetus fetus*, *C. jejuni*, *C. pylori* (*Helicobacter pylori*); *Vibrio*, e.g., *V. cholerae*, *V. parahaemolyticus*, *V. mimicus*, *V. alginolyticus*, *V. hollisae*, *V. vulnificus*, and the nonagglutinable vibrios; *Clostridia*, e.g., *C. perfringens*, *C. tetani*, *C. difficile*, *C. botulinum*; *Aetionomyces*, e.g., *A. israelii*, *Bacteroides*, e.g., *B. fragilis*, *B. thetaiotaomicron*, *B. distasonis*, *B. vulgatus*, *B. ovatus*, *B. caccae*, and *B. merdae*; *Prevotella*, e.g., *P. melaninogenica*; genus *Fusobacterium*; *Treponema*, e.g., *T. pallidum* subspecies *endemicum*, *T. pallidum* subspecies *pertenue*, *T. carateum*, and *T. pallidum* subspecies *pallidum*; genus *Borrelia*, e.g., *B. burgdorferi*; genus *Leptospira*; *Streptobacillus*, e.g., *S. moniliformis*; *Spirillum*, e.g., *S. minus*; *Mycobacterium*, e.g., *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. xenopi*, *M. marinum*, *M. ulcerans*, the *M. fortuitum* complex (*M. fortuitum* and *M. chelonae*), *M. leprae*, *M. asiaticum*, *M. chelonae* subsp. *abscessus*, *M. fallax*, *M. fortuitum*, *M. malmoeense*, *M. shimoidei*, *M. simliae*, *M. szulgai*, *M. xenopi*; *Mycoplasma*, e.g., *M. hominis*, *M. orale*, *M. salivarium*, *M. fermentans*, *M. pneumoniae*, *M. bovis*, *M. avium*, *M. leprae*; *Mycoplasma*, e.g., *M. genitalium*; *Ureaplasma*, e.g., *U. urealyticum*; *Trichomonas*, e.g., *T. vaginalis*; *Cryptococcus*, e.g., *C. neoformans*; *Histoplasma*, e.g., *H. capsulatum*; *Candida*, e.g., *C. albicans*; *Aspergillus* sp.; *Coccidioides*, e.g., *C. immitis*; *Blastomyces*, e.g., *B. dermatitidis*; *Paracoccidioides*, e.g., *P. brasiliensis*; *Penicillium*, e.g., *P. marneffei*; *Sporothrix*, e.g., *S. schenckii*; *Rhizopus*, *Rhizomucor*, *Absidia*, and *Basidiobolus*; diseases caused by *Bipolaris*, *Cladophialophora*, *Cladosporium*, *Drechslera*, *Exophiala*, *Fonsecaea*, *Phialophora*, *Xylohypha*, *Ochroconis*, *Rhinocladiella*, *Scolecobasidium*, and *Wangiella*; *Trichosporon*, e.g., *T. beigellii*; *Blastoschizomyces*, e.g., *B. capitatus*; *Plasmodium*, e.g., *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*; *Babesia* sp.; protozoa of the genus *Trypanosoma*, e.g., *T. cruzi*; *Leishmania*, e.g., *L. donovani*; *L. major*, *L. tropica*, *L. mexicana*, *L. braziliensis*, *L. viannia braziliensis*; *Toxoplasma*, e.g., *T. gondii*; Amoebas of the genera *Naegleria* or *Acanthamoeba*; *Entamoeba histolytica*; *Giardia lamblia*; genus *Cryptosporidium*, e.g., *C. parvum*; *Isospora belli*; *Cyclospora cayentanensis*; *Ascaris lumbricoides*; *Trichuris trichiura*; *Ancylostoma duodenale* or *Necator americanus*; *Strongyloides stercoralis* *Toxocara*, e.g., *T. canis*, *T. cati*; *Baylisascaris*, e.g., *B. procyonis*; *Trichinella*, e.g., *T. spiralis*; *Dracunculus*, e.g., *D. medinensis*; genus *Filarioidea*; *Wuchereria bancrofti*; *Brugia*, e.g., *B. malayi*, or *B. timori*; *Onchocerca volvulus*; *Loa loa*; *Dirofilaria immitis*; genus *Schistosoma*, e.g., *S. japonicum*, *S. mansoni*, *S. mekongi*, *S. intercalatum*, *S. haematobium*; *Paragonimus*, e.g., *P. westermani*, *P. skrjabini*; *Clonorchis sinensis*; *Fasciola hepatica*; *Opisthorchis* sp.; *Fasciolopsis buski*; *Diphyllbothrium latum*; *Taenia*, e.g., *T. saginata*, *T. solium*; *Echinococcus*, e.g., *E. granulosus*, *E. multilocularis*; Picornaviruses, rhinoviruses, echoviruses, coxsackieviruses, influenza virus; paramyxoviruses, e.g., types 1, 2, 3, and 4; adenoviruses; Herpesviruses, e.g., HSV-1 and HSV-2; varicella-zoster virus; human T-lymphotrophic virus (type I and type II); *Arboviruses* and

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Arenaviruses; *Togaviridae*, *Flaviviridae*, *Bunyaviridae*, *Reoviridae*; *Flavivirus*; *Hantavirus*; *Viral encephalitis* (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis]); *Viral hemorrhagic fevers* (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo]); *Smallpox* (variola); retroviruses e.g., human immunodeficiency viruses 1 and 2; human papillomavirus [HPV] types 6, 11, 16, 18, 31, 33, and 35.

10 In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter Baumannii*, *Serratia marcescens*, *Enterobacter aerogenes*, *Enterococcus faecium*, vancomycin-resistant enterococcus (VRE), *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus viridans*, *Listeria monocytogenes*, *Enterococcus* spp., *Streptococcus* 15 Group B, *Streptococcus* Group C, *Streptococcus* Group D, *Streptococcus* Group F, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Gardnerella vaginalis*, *Micrococcus* spp., *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Moraxella catarrhalis*, *Salmonella* spp., *Chlamydia trachomatis*, *Peptostreptococcus productus*, *Peptostreptococcus anaerobius*, *Lactobacillus fermentum*, *Eubacterium lentum*, *Candida glabrata*, *Candida albicans*, *Chlamydia* spp., *Campylobacter* spp., *Salmonella* spp., smallpox (variola major), *Yersinia Pestis*, *Herpes Simplex Virus I* (HSV I), and *Herpes Simplex Virus II* (HSV II).

20 In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of Group B *Streptococcus*.

25 Carrying out PCR on a PCR-ready sample can include heating the PCR reagent mixture and the neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample; contacting the neutralized polynucleotide sample or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; independently 30 contacting each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide; and/or contacting the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide 35 sequence.

40 In various embodiments, a method of carrying out PCR on a sample can further include one or more of the following steps: heating the biological sample in the microfluidic cartridge; pressurizing the biological sample in the microfluidic cartridge at a pressure differential compared to ambient pressure of between about 20 kilopascals and 200 kilopascals, or in some embodiments between about 70 kilopascals and 110 kilopascals.

45 In various embodiments, a method of using the apparatus described herein can further include one or more of the following steps: determining the presence of a polynucleotide sequence in the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; determining a contaminated result if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof; and/or in some embodiments, wherein the 50

PCR reagent mixture further comprises a positive control plasmid and a plasmid probe selective for at least a portion of the plasmid, the method further including determining a PCR reaction has occurred if the plasmid probe is detected. Fluorescence Detection System, Including Lenses and Filters, and Multiple Parallel Detection for a Multi-Lane Cartridge

A miniaturized, highly sensitive fluorescence detection system can be incorporate for monitoring fluorescence from the biochemical reactions that are the basis of nucleic acid amplification methods such as PCR.

Accordingly, another aspect of the apparatus includes a system for monitoring fluorescence from biochemical reactions. The system can be, for example, an optical detector having a light source (for example an LED) that selectively emits light in an absorption band of a fluorescent dye, lenses for focusing the light, and a light detector (for example a photodiode) that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. Alternatively, the optical detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye (a fluorogenic probe) and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes at a plurality of different locations of, for example, a microfluidic cartridge, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof.

In some embodiments, a given detector for use with the apparatus described herein is capable of detecting a fluorescence signal from nanoliter scale PCR reactions. Advantageously, the detector is formed from inexpensive components, having no moving parts. The detector is also configured to mate with a microfluidic cartridge as further described herein, and is also preferably part of a pressure application system, such as sliding lid, that keeps the cartridge in place. The detector further has potential for 2 or 3 color detection and is controlled by software, preferably custom software, configured to sample information from the detector.

FIGS. 57-59 depict an embodiment of a highly sensitive fluorescence detection system including light emitting diodes (LED's), photodiodes, and filters/lenses for monitoring, in real-time, one or more fluorescent signals emanating from the microfluidic cartridge. The embodiment in FIGS. 57-59 has a two-color detection system having a modular design that mates with a single lane microfluidic cartridge. The detector comprises two LED's (blue and red, respectively) and two photodiodes. The two LED's are configured to transmit a beam of focused light on to a particular region of the cartridge. The two photodiodes are configured to receive light that is emitted from the region of the cartridge. One photodiode is configured to detect emitted red light, and the other photodiode is configured to detect emitted blue light.

FIGS. 60 and 61 show an exemplary read-head comprising a multiplexed 2 color detection system, such as multiple instances of a detection system shown in FIGS. 57-59, that is configured to mate with a multi-lane microfluidic cartridge. FIG. 60 shows a view of the exterior of a multiplexed

read-head. FIG. 61 is an exploded view that shows how various detectors are configured within an exemplary multiplexed read head, and in communication with an electronic circuit board.

The module in FIGS. 60 and 61 is configured to detect fluorescence from each lane of a 12-lane cartridge, and therefore comprises 24 independently controllable detectors, arranged as 12 pairs of identical detection elements. Each pair of elements is then capable of dual-color detection of a pre-determined set of fluorescent probes. It would be understood by one of ordinary skill in the art that other numbers of pairs of detectors are consistent with the apparatus described herein. For example, 4, 6, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 pairs are also consistent and can be configured according to methods and criteria understood by one of ordinary skill in the art.

Exemplary Optics Assembly

In an exemplary embodiment, the optical chassis/pressure assembly is housed in an enclosure (made of plastic in certain embodiments) that can be positioned to cover a multi-lane microfluidic cartridge. The enclosure can optionally have a handle that can be easily grasped by a user, and is guided for smooth and easy pushing and pulling. The handle may also serves as a pressure-locking device. The enclosure's horizontal position is sensed in both the all-open and in the all-forward position, and reported to controlling software. The enclosure and optical chassis pressure assembly registers with a heater cassette module positioned underneath a microfluidic cartridge to within 0.010". A close fit is important for proper heater/cartridge interface connections. The enclosure assembly does not degrade in performance over a life of 10,000 cycles, where a cycle is defined as: beginning with the slider in the back position, and sliding forward then locking the handle down on a cartridge, unlocking the handle and returning it to the original back position. All optical path parts should be non-reflective (anodized, painted, molded, etc.) and do not lose this feature for 10,000 cycles. The optics unit is unaffected by a light intensity of $\leq 9,000$ foot-candles from a source placed 12" from the instrument at angles where light penetration is most likely to occur. No degradation of performance is measured at the photo-detector after 10,000 cycles.

When fabricating a detector assembly, a single channel is made that houses two LED sources (blue and amber) and two additional channels that house one photodiode detector each (four total bored holes). The two paired channels (source and detector) are oriented 43° from each other, measured from the optical axis and are in-line with the other paired channels that are at the same 43° orientation. The holes bored in the optical chassis contain filters and lenses with appropriate spacers, the specification of which are further described herein. The LED's are held in place to prevent movement as the mechanical alignment is important for good source illumination. The LED's are preferably twisted until the two "hot spots" are aligned with the reading channels on the cartridge. This position must be maintained until the LED's cannot be moved. The optical chassis can be made of aluminum and be black anodized. The bottom pressure surface of the optical chassis is flat to ± 0.001 " across the entire surface. The optical chassis is center-balanced such that the center of the optical chassis force is close to the center of the reagent cartridge. The pressure assembly (bottom of the optical chassis) provides uniform pressure of a minimum of 1 psi across all heater sections of the reagent cartridge. The optical assembly can be moved away from the reagent cartridge area for cartridge removal

and placement. Appropriate grounding of the optical chassis is preferred to prevent spurious signals to emanate to the optic PCB.

The LED light sources (amber and blue) are incident on a microfluidic cartridge through a band pass filter and a focusing lens. These LED light sources have a minimum output of 2800 millicandles (blue) and 5600 millicandles (Green), and the center wavelengths are 470 (blue) and 575 (amber) nanometers, with a half band width of no more than 75 nanometers.

The LED light excites at least one fluorescent molecule (initially attached to an oligonucleotide probe) in a single chamber on a cartridge, causing it to fluoresce. This fluorescence will normally be efficiently blocked by a closely spaced quencher molecule. DNA amplification via TAQ enzyme will separate the fluorescent and quenching molecules from the oligonucleotide probe, disabling the quenching. DNA amplification will only occur if the probe's target molecule (a DNA sequence) is present in the sample chamber. Fluorescence occurs when a certain wavelength strikes the target molecule. The emitted light is not the same as the incident light. Blue incident light is blocked from the detector by the green only emission filter. Green incident light similarly is blocked from the detector by the yellow emission filter. The fluorescent light is captured and travels via a pathway into a focusing lens, through a filter and onto a very sensitive photodiode. The amount of light detected increases as the amount of the DNA amplification increases. The signal will vary with fluorescent dye used, but background noise should be less than 1 mV peak-to-peak. The photo-detector, which can be permanently mounted to the optical chassis in a fixed position, should be stable for 5 years or 10,000 cycles, and should be sensitive to extremely low light levels, and have a dark value of no more than 60 mV. Additionally, the photo-detector must be commercially available for at least 10 years. The lenses are Plano-convex (6 mm detector, and 12 mm source focal length) with the flat side toward the test cartridge on both lenses. The filters should remain stable over normal operating humidity and temperature ranges.

The filters, e.g., supplied by Omega Optical (Braunelboro, Vt. 05301), are a substrate of optical glass with a surface quality of F/F per Mil-C-48497A. The individual filters have a diameter of 6.0 ± 0.1 mm, a thickness of 6.0 ± 0.1 mm, and the AOI and $\frac{1}{2}$ cone AOI is 0 degrees and ± 8 degrees, respectively. The clear aperture is ≥ 4 mm diameter and the edge treatment is blackened prior to mounting in a black, anodized metal ring. The FITC exciter filters is supplied by, e.g., Omega Optical (PN 481AF30-RED-EXC). They have a cut-off frequency of 466 ± 4 nm and a cut-on frequency of 496 ± 4 nm. Transmission is $\geq 65\%$ peak and blocking is: $\geq OD8$ in theory from 503 to 580 nm, $\geq OD5$ from 501-650 nm, $\geq OD4$ avg. over 651-1000 nm, and $\geq OD4$ UV-439 nm. The FITC emitter filters is supplied by, e.g., Omega Optical (PN 534AF40-RED-EM). They will have a cut-off frequency of 514 ± 2 nm and a cut-on frequency of 554 ± 4 nm. Transmission is $\geq 70\%$ peak and blocking is: $\geq OD8$ in theory from 400 to 504 nm, $\geq OD5$ UV-507 nm, and $\geq OD4$ avg. 593-765 nm. The amber exciter filters are supplied by, e.g., Omega Optical (PN 582AF25-RED-EXC). They have a cut-off frequency of 594 ± 5 nm and a cut-on frequency of 569 ± 5 nm. Transmission is $\geq 70\%$ peak and blocking is: $\geq OD8$ in theory from 600 to 700 nm, $\geq OD5$ 600-900 nm, and $\geq OD4$ UV-548 nm. The amber emitter filters are supplied by, e.g., Omega Optical (PN 627AF30-RED-EM). They have a cut-off frequency of 642 ± 5 nm and a cut-on frequency of 612 ± 5 nm. Transmission is $\geq 70\%$

peak and blocking is: $\geq OD8$ in theory from 550 to 600 nm, $\geq OD5$ UV-605 nm, and $\geq OD5$ avg. 667-900 nm. The spacers should be inert and temperature stable throughout the entire operating range and should maintain the filters in strict position and alignment. The epoxy used should have optically black and opaque material and dry solid with no tacky residue. Additionally, it should have temperature and moisture stability, exert no pressure on the held components, and should mount the PCB in such a way that it is fixed and stable with no chances of rotation or vertical height changes. 50% of illumination shall fall on the sample plane within an area 0.1" (2.5 mm) wide by 0.3" (7.5 mm) along axis of the detection channel. Fluorescence of the control chip should not change more than 0.5% of the measured signal per 0.001" of height though a region ± 0.010 from the nominal height of the control chip.

An exemplary optics board is shown in FIG. 62, and is used to detect and amplify the fluorescent signature of a successful chemical reaction on a micro-fluidic cartridge, and controls the intensity of LED's using pulse-width modulation (PWM) to illuminate the cartridge sample over up to four channels, each with two color options. Additionally, it receives instructions and sends results data back over an LVDS (low-voltage differential signaling) SPI (serial peripheral interface). The power board systems include: a +12V input, and +3.3V, +3.6V, +5V, and -5V outputs, configured as follows: the +3.3V output contains a linear regulator, is used to power the LVDS interface, should maintain a $\pm 5\%$ accuracy, and supply an output current of 0.35 A; the +3.6V output contains a linear regulator, is used to power the MSP430, should maintain a $\pm 5\%$ accuracy, and supply an output current of 0.35 A; the +5V output contains a linear regulator, is used to power the plus rail for op-amps, should maintain a $\pm 5\%$ accuracy, and supply an output current of 0.35 A; the -5V output receives its power from the +5V supply, is used to power the minus rail for op-amps and for the photo-detector bias, should maintain a $\pm 1\%$ voltage accuracy, and supply an output current of 6.25 mA $\pm 10\%$. Additionally, the power board has an 80 ohm source resistance, and the main board software can enable/disable the regulator outputs.

The main board interface uses a single channel of the LVDS standard to communicate between boards. This takes place using SPI signaling over the LVDS interface which is connected to the main SPI port of the control processor. The interface also contains a serial port for in-system programming.

The exemplary optical detection system of FIG. 62 consists of a control processor, LED drivers, and a photo-detection system. In the exemplary embodiment, the control processor is a TI MSP430F1611 consisting of a dual SPI (one for main board interface and one for ADC interface) and extended SRAM for data storage. It has the functions of power monitoring, PWM LED control, and SPI linking to the ADC and main board. The LED drivers contain NPN transistor switches, are connected to the PWM outputs of the control processor, can sink 10 mA @ 12V per LED (80 mA total), and are single channel with 2 LEDs (one of each color) connected to each. The photo-detection system has two channels and consists of a photo-detector, high-sensitivity photo-diode detector, high gain current to voltage converter, unity gain voltage inverting amplifier, and an ADC. Additionally it contains a 16 channel Sigma-delta (only utilizing the first 8 channels) which is connected to the second SPI port of the control processor. It would be understood by one of ordinary skill in the art that other

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choices and combinations of elements can be brought together to make a functioning detection system consistent with the description herein.

Additional Advantages and Features of the Technology Herein

The use of a disposable process chamber, having surface coating and material properties to allow low volume, and open tube heated release to maximize sample concentration in lowest volume possible.

The integrated magnetic heat separator that allows multiple samples to be heated independently but separated using a single moveable magnet platform.

A reader/tray design that allows easy placement of microfluidic cartridge and multiple sample pipetting of liquid using a robotic dispenser in one position; relative displacement to another location and pressure application for subsequent rapid heat incubation steps and optical detection. The bottom surface of the cartridge mates with the heating surface. Furthermore, it is typically easier to move a cartridge and heater in and out of position than a detector.

A moveable readhead design for fluorescence detection from microfluidic PCR channels.

Aspects of the holder, such as a unitized disposable strip, that include the presence of sealed lyophilized reagents as well as liquids sealed in close proximity, which is normally hard to achieve. The laminates deployed herein make storage easier.

The holder permits snapping of multiple ASR tubes, and associated liquid dispensing processes that minimizes cross-sample contamination but multiple PCR preparations to be performed from a single clinical sample.

Software features allow a user to either get results from all 24 samples as quickly as possible or the first 12 samples as quickly as possible and the next 12 later.

The preparatory and diagnostic instruments described herein enables different sample types (such as blood, urine, swab, etc.) to be all processed at the same time even though each may require different temperatures, times or chemical reagents. This is achieved in part by using individualized but compatible holders.

Automatic feeding of microfluidic cartridges into a PCR reader via a cartridge autoloader saves a user time and leads to increased efficiency of overall operation.

Piercing through foil over a liquid tube and reliable way of picking up liquid.

A moveable read-head that has the pumps, sensors (pipette detection, force sensing), sample identification verifier, etc., moving with it, and therefore minimizes the number of control lines that move across the instrument during use.

Accurate and rapid alignment of pipette tips with cartridge inlet holes using a motorized alignment plate.

EXAMPLES

Example 1: Reagent Holder

An exemplary reagent holder consistent with the description herein has the following dimensions and capacities:

180 mm long×22 mm wide×100 mm tall;

Made from Polypropylene.

One snapped-in low binding 1.7 ml tube that functions as a process tube.

3 built-in tubes that function as receptacles for reagents, as follows:

One tube containing 200-1000 μ l of wash buffer (0.1 mM Tris, pH 8).

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One tube containing 200-1000 μ l of release solution (40 mM NaOH).

One tube containing 200-1000 μ l of neutralization solution (330 mM Tris, pH 8.0).

One built-in tube that functions as a waster chamber (will hold ~4 ml of liquid waste).

3 receptacles to accept containers for solid reagents. Snap-in 0.3 ml or 0.65 ml PCR tubes (which are typically stored separately from the reagent holder) are placed in each of these locations, and contain, respectively;

lyophilized sample preparation reagents (lysis enzyme mix and magnetic affinity beads).

First lyophilized PCR master mix, probes and primers for a first target analyte detection.

Second lyophilized PCR master mix, probes and primers for a second target analyte detection (only offered in select cases, such as detection of Chlamydia and Gonorrhea from urine).

4 pipette tips located in 4 respective sockets.

Pipette tip Sheath: The pipette tips have a sheath/drip tray underneath to help capture any drip from the pipette tips after being used, and also to prevent unwanted contamination of the instrument.

Handle and Flex-Lock allows easy insertion, removal, and positive location of strip in rack.

One or more labels: positioned upward facing to facilitate ease of reading by eye and/or, e.g., a bar-code reader, the one or more labels containing human and machine readable information pertaining to the analysis to be performed.

It is to be understood that these dimension are exemplary. However, it is particularly desirable to ensure that a holder does not exceed these dimensions so that a rack and an apparatus that accommodates the reagent holder(s) does not become inconveniently large, and can be suitably situated in a laboratory, e.g., on a bench-top.

Example 2: Disposable Reagent Holder Manufacturing

Simple fixtures can be designed and machined to enable handling and processing of multiple strips. These are five steps that can be performed to produce this component. The disposable reagent holder will be placed in a fixture and filled with liquids using manual/electric-multiple pipetting. Immediately after dispensing all liquids into the strip, foil will be heat sealed to the plastic using exemplary heat seal equipment (Hix FH-3000-D Flat Head Press) and the foil trimmed as required. After heat sealing liquids on board, all pellets in tubes can be snapped into the strip, pipette tips can be inserted in their respective sockets, and a barcode label can be affixed. Desiccant packs can be placed into the blow molded or thermoformed rack designed to house 12 holders. Twelve disposable strips will be loaded into the rack and then sealed with foil. The sealed bag will be placed into a carton and labeled for shipping.

Example 3: Foil-Sealing of Buffer Containing Reagent Tubes

Tubes containing buffers have to be sealed with high moisture vapor barrier materials in order to retain the liquid over a long period of time. Disposable holders may need to have a shelf life of 1-2 years, and as such, they should not lose more than say 10-15% of the liquid volume over the time period, to maintain required volume of liquid, and to

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maintain the concentration of various molecules present in the solution. Moreover, the materials used for construction of the tube as well as the sealing laminate should not react with the liquid buffer. Special plastic laminates may provide the moisture barrier but they may have to be very thick (more than 300 μm thick), causing the piercing force to go up tremendously, or of special, expensive polymer (such as Aclar). Aluminum foils, even a thin foil of a few hundred angstrom provides an effective moisture barrier but bare aluminum reacts with some liquid buffers, such as sodium hydroxide, even an aluminum foil with a sprayed coating of a non-reactive polymer may not be able to withstand the corrosive vapors over a long time. They may react through tiny pin holes present in the coating and may fail as a barrier over time.

For these reasons, aluminum foils with a laminate structure have been identified as a suitable barrier, exemplary properties of which are described below:

1. Sealing

Heat seals to unitized polypropylene strip (sealing temp $\sim 170\text{--}180^\circ\text{C}$.)

No wrinkling, cracking and crazing of the foil after sealing

2. Moisture Vapor Transmission Rate (MVTR)

Loss of less than 10% liquid (20 microliters from a volume of 200 microliter) for a period of 1 year stored at ambient temperature and pressure (effective area of transport is $\sim 63\text{ mm}^2$); Approximate MVTR $\sim 0.8\text{ cc/m}^2/\text{day}$

3. Chemistry

Ability to not react with 40 mM Sodium Hydroxide ($\text{pH} < 12.6$): foil should have a plastic laminate at least 15 microns thick closer to the sealed fluid.

Ability to not react with other buffers containing mild detergents

4. Puncture

Ability to puncture using a p1000 pipette with a force less than 3 lb

Before puncturing, a fully supported membrane 8 mm in diameter will not stretch more than 5 mm in the orthogonal direction

After puncturing, the foil should not seal the pipette tip around the circumference of the pipette.

5. Other Features

Pin-hole free

No bubbles in case of multi-laminate structures.

Example 4: Mechanism of Piercing through a Plasticized Laminate and Withdrawing Liquid Buffer

The aluminum laminate containing a plastic film described elsewhere herein serves well for not reacting with corrosive reagents such as buffers containing NaOH, and having the favorable properties of pierceability and acting as a moisture barrier. However, it presents some additional difficulties during piercing. The aluminum foil tends to burst into an irregular polygonal pattern bigger than the diameter of the pipette, whereas the plastic film tends to wrap around the pipette tip with minimal gap between the pipette and the plastic film. The diameter of the hole in the plastic film is similar to the maximum diameter of the pipette that has crossed through the laminate. This wrapping of the pipette causes difficulty in dispensing and pipetting operations unless there is a vent hole allowing pressures to equilibrate between outside of the tube and the air inside of the tube.

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A strategy for successful pipetting of fluid is as follows:

1. Pierce through the laminate structure and have the pipette go close to the bottom of the reagent tube so that the hole created in the laminate is almost as big as the maximum diameter of the pipette (e.g., $\sim 6\text{ mm}$ for a p1000 pipette)
2. Withdraw the pipette up a short distance so that a small annular vent hole is left between the pipette and the laminate. The p1000 pipette has a smallest outer diameter of 1 mm and maximum outer diameter of 6 mm and the conical section of the pipette is about 28 mm long. A vent hole thickness of a hundred microns is enough to create a reliable vent hole. This corresponds to the pipette inserted to diameter of 5.8 mm, leaving an annulus of 0.1 mm around it.
3. Withdraw fluid from the tube. Note that the tube is designed to hold more fluid than is necessary to withdraw from it for a sample preparation procedure.

Example 5: Foil Piercing and Dissolution of Lyophilized Reagents

The containers of lyophilized reagents provided in conjunction with a holder as described herein are typically sealed by a non-plasticized aluminum foil (i.e., not a laminate as is used to seal the reagent tubes). Aluminum foil bursts into an irregular polygonal pattern when pierced through a pipette and leaves an air vent even though the pipette is moved to the bottom of the tube. In order to save on reagents, it is desirable to dissolve the reagents and maximize the amount withdrawn from the tube. To accomplish this, a star-ridged (stellated) pattern is placed at the bottom of the container to maximize liquid volume withdrawn, and flow velocity in between the ridges.

Exemplary steps for dissolving and withdrawing fluid are as follows:

1. Pierce through the pipette and dispense the fluid away from the lyophilized material. If the pipette goes below the level of the lyophilized material, it will go into the pipette and may cause jamming of the liquid flow out of the pipette.
2. Let the lyophilized material dissolve for a few seconds.
3. Move pipette down touching the ridged-bottom of the tube
4. Perform an adequate number of suck and spit operations (4-10) to thoroughly mix the reagents with the liquid buffer.
5. Withdraw all the reagents and move pipette to dispense it into the next processing tube.

Example 6: Material and Surface Property of the Lysis Tube

The material, surface properties, surface finish has a profound impact on the sensitivity of the assay performed. In clinical applications, DNA/RNA as low as 50 copies/sample ($\sim 1\text{ ml}$ volume) need to be positively detected in a background of billions of other molecules, some of which strongly inhibit PCR. In order to achieve these high level of sensitivities, the surface of the reaction tube as well as the material of the surface has to be chosen to have minimal binding of polynucleotides. During the creation of the injection molding tool to create these plastic tubes, the inherent surfaces created by machining may have large surface area due to cutting marks as large as tens of microns of peaks and valleys. These surfaces have to be polished to SPI A1/A2 finish (mirror finish) to remove the microscopic surface irregularities. Moreover, the presence of these microscopic

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valleys will trap magnetic heads (0.5-2 μ) at unintended places and cause irregular performance. In addition to actual surface roughness, the surface hydrophobicity/surface molecules present may cause polynucleotides to stick at unintended places and reduce sensitivity of the overall test. In addition to the base material uses, such as homogenous polupropylene and other polymers, specific materials used during the molding of these tubes, such as mold release compounds or any additives to aid in the fabrication can have a profound impact on the performance of the reactions.

Example 7: Liquid Dispensing Head

Referring to FIGS. 18, 19A-C, and 63, an exemplary liquid dispenser is attached to a gantry, and receives instructions via electrical cable 1702. Barcode scanner 1701 is mounted on one face of the liquid dispenser. The gantry is mounted on a horizontal rail 1700 to provide movement in the x-direction. Not shown is an orthogonally disposed rail to provide movement in the y-direction. The liquid dispenser comprises a computer controlled motorized pump 1800 connected to fluid distribution manifold 1802 with related computer controlled valving 1801 and a 4-up pipettor with individually sprung heads 1803. The fluid distribution manifold has nine Lee Co. solenoid valves 1801 that control the flow of air through the pipette tips: two valves for each pipette, and an additional valve to vent the pump. Barcode reader 1701 enables positive detection of sample tubes, reagent disposables and microfluidic cartridges. The scanner is mounted to the z-axis so that it can be positioned to read the sample tube, strip, and cartridge barcodes.

Example 8: Integrated Heater/Separator

In FIG. 64 an exemplary integrated magnetic separator and heater assembly are shown. Magnetic separator 1400 and heater assembly 1401 were fabricated comprising twelve heat blocks aligned parallel to one another. Each heat block 1403 is made from aluminum, and has an L-shaped configuration having a U-shaped inlet for accepting a process chamber 1402. Each heat block 1403 is secured and connected by a metal strip 1408 and screws 1407. Magnet 1404 is a rectangular block Neodymium (or other permanent rare earth materials, K & J Magnetics, Forcefield Magnetics) disposed behind each heat block 1403 and mounted on a supporting member. Gears 1406 communicate rotational energy from a motor (not shown) to cause the motorized shaft 1405 to raise and lower magnet 1404 relative to each heat block. The motor is computer-controlled to move the magnet at speeds of 1-20 mm/s. The device further comprises a printed circuit board (PCB) 1409 configured to cause the heater assembly to apply heat independently to each process chamber 1402 upon receipt of appropriate instructions. In the exemplary embodiment, the device also comprises a temperature sensor and a power resistor in conjunction with each heater block.

Example 9: Exemplary Software

Exemplary software accompanying use of the apparatus herein can include two broad parts—user interface and device firmware. The user interface software can allow for aspects of interaction with the user such as—entering patient/sample information, monitoring test progress, error warnings, printing test results, uploading of results to databases and updating software. The device firmware can be the low level software that actually runs the test. The firmware

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can have a generic portion that can be test independent and a portion specific to the test being performed. The test specific portion (“protocol”) can specify the microfluidic operations and their order to accomplish the test.

FIGS. 64A and 65B shows screen captures from the programming interface and real time heat sensor and optical detector monitoring. This real time device performance monitoring is for testing purposes; not visible to the user in the final configuration.

User Interface:

A medical grade LCD and touch screen assembly can serve as the user interface via graphical user interface providing easy operating and minor troubleshooting instructions. The LCD and touch screen have been specified to ensure compatibility of all surfaces with common cleaning agents. A barcode scanner integrated with the analyzer can be configured to scan the barcode off the cartridge (specifying cartridge type, lot #, expiry date) and if available the patient and user ID from one or more sample tubes.

Example 10: Exemplary Preparatory Apparatus

This product is an instrument that enables 24 clinical samples to be automatically processed to produce purified nucleic acid (DNA or RNA) in about half an hour (FIG. 66). Purified nucleic acid may be processed in a separate amplification-detection machine to detect the presence of certain target nucleic acids. Samples are processed in a unitized disposable strip, preloaded with sample preparation chemistries and final purified nucleic acids are dispensed into PCR tubes. Fluid handling is enabled by a pipetting head moved by a xyz gantry. (FIG. 67)

The System has the following sub-systems:

- Two sample processing racks, each rack processes up to 12 clinical samples in unitized disposable strips
- Magnetic separator-cum-tube heater assembly (24 heating stations)

- A four-probe liquid dispensing head

- 3-axis gantry to move the pipette head

- Peltier-cooled per-tube holding station to receive the purified DNA/RNA

- Control electronics

- Barcode reader

Operation: The user will get a work list for each sample, whether they want to extract DNA or RNA for each clinical sample. The sample tubes are placed on the rack and for each sample type (DNA or RNA), the user slides in a unitized reagent disposable (DNA or RNA processing) into corresponding lane of the rack. The unitized disposable (holder) will have all the sample prep reagents, process tubes as well as disposable pipettes already prepackaged in it. Once all disposables are loaded into the rack, the rack is placed in its location on the instrument. Open per tubes are placed in the peltier cooled tube holder where the final purified nucleic acid will be dispensed. The user then closes the door of the instrument and then starts the sample processing using the GUI (Graphical User Interface).

The instrument checks functionality of all subsystems and then reads the barcode of the sample tubes and the unitized reagent disposable. Any mismatch with a pre-existing work list is determined and errors are flagged, if necessary. The instrument then goes through a series of liquid processing, heating, magnetic separations to complete the sample preparation steps for the each of the clinical sample and outputs the purified nucleic acid into the PCR tube. The basic steps involved in each sample processing are sample lysis, nucleic acid capture into magnetic affinity beads, washing of the

magnetic beads to remove impurities, releasing the nucleic acid from the magnetic beads, neutralizing the released DNA and the dispensing into the final PCR tube. These tubes are maintained at 4° C. until all samples are processed and user takes away the tube for downstream processing of the nucleic acids.

Example 11: Exemplary Diagnostic Apparatus

The apparatus, in combination with the associated consumables, automatically performs all aspects of nucleic acid testing, including sample preparation, amplification, and detection for up to 48 samples per hour with the first 24 results available in less than an hour. The system is easy to use. An operator simply aliquots a portion of the patient sample into a dedicated tube that contains pre-packaged buffer. The operator places the dedicated tubes into positions on a sample rack. The operator then loads a disposable plastic reagent strip for the appropriate test in the rack. The only other consumable used in the apparatus are microfluidic PCR cartridges for conducting amplification and detection; each cartridge is capable of performing up to twelve PCR tests and two cartridges can be loaded into the analyzer at once. Should the apparatus require a new PCR cartridge, the analyzer will prompt the operator to load the cartridge. The analyzer will then prompt the operator to close the lid to initiate testing. All consumables and sample tubes are bar-coded for positive sample identification.

Sample lysis and DNA preparation, which will require approximately half an hour for a full run of 24 samples, is automatically performed by the analyzer's robotic and liquid handling components using protocols and reagents located in unitized, disposable plastic strips. The apparatus then automatically mixes the samples and PCR reagents, and injects the mixture into a cartridge that will be automatically processed by an integrated PCR machine. Rapid, real time PCR and detection requires less than 20 minutes. Results, which will be automatically available upon completion of PCR, are displayed on the instrument's touch screen, printed or sent to the hospital information system, as specified by the user (or the user's supervisor).

Each instrument can process up to 24 samples at a time with a total throughput of 48 samples per hour after the first run. The analyzer is slightly less than 1 m wide and fits easily on a standard lab bench. All operations of the unit can be directed using the included barcode wand and touch screen. The analyzer can be interfaced with lab information systems, hospital networks, PCs, printers or keyboards through four USB interfaces and an Ethernet port.

The apparatus has the following characteristics:

Sensitivity: the apparatus will have a limit of detection of ~50 copies of DNA or RNA. (and may have a limit of detection as low as 25-30 copies of DNA/RNA).

Cost per Test: Due to the miniaturized, simplified nature of HandyLab reagents, cartridge and other consumables, the cost of goods per test will be relatively low and very competitive.

Automation: By contrast with current "automated" NAT systems, which all require some degree of reasonably extensive technologist interaction with the system, through the use of unitized tests and full integration of sample extraction, preparation, amplification and detection, the apparatus herein will offer a higher level of automation, and corresponding reduction in technologist time and required skill level, thereby favorably impacting overall labor costs.

Throughput: Throughput is defined as how many tests a system can conduct in a given amount of time. The apparatus will be capable of running 45 tests per hour, on average.

Time to First Result: In a hospital environment, time to first result is an especially important consideration. The apparatus will produce the first 24 results in less than an hour and an additional 24 results every half hour thereafter.

Random Access and STAT: Random access is the ability to run a variety of tests together in a single run and place samples in unassigned locations on the analyzer. Also, with chemistry and immunoassay systems, it is desirable to be able to add test after a run has started. This is often referred to as "true random access" since the user is provided complete flexibility with regard to what tests can be run where on an analyzer and when a new sample can be added to a run. A STAT is a sample that requires as rapid a result as possible, and therefore is given priority in the testing cue on the analyzer. Today, essentially all chemistry and immunoassay analyzers are true random access and offer STAT capabilities. For NAT, however, very few systems offer any random access or STAT capabilities. The instrument herein will provide random access and STAT capabilities.

Menu: The number and type of tests available for the analyzer is a very important factor in choosing systems. The apparatus herein deploys a launch menu strategy that involves a mix of high volume, "standard" nucleic acid tests combined with novel, high value tests.

The apparatus enables 24 clinical samples to be automatically processed to purify nucleic acid, mix the purified DNA/RNA with PCR reagents and perform real-time PCR in microfluidic cartridge to provide sample to results in an hour. The exemplary apparatus has two PCR readers, each capable of running a 12 lane microfluidic cartridge using an optical system that has dedicated two-color optical detection system. FIG. 68, FIG. 69.

The apparatus has the following sub-systems:

- Two sample processing rack, each rack processes up to 12 clinical samples in unitized disposable strips.

- Magnetic separator-cum-tube heater assembly (24 heating stations)

- A four-probe liquid dispensing head

- 3-axis gantry to move the pipette head

- Two PCR amplification-detection station, each capable of running a 12-lane microfluidic cartridge and dedicated 2-color optical detection system for each PCR lane.

- Control electronics

- Barcode reader

Pictures of exterior (face on) and interior are at FIGS. 70, 71, respectively.

Operation: The user will get a work list for each sample, whether they want to detect certain target analyte (such as GBS, Chlamydia, Gonorrhea, HSV) for each clinical sample. The sample tubes are placed on the rack and for each sample, the user slides in a unitized reagent disposable (analyte specific) into corresponding lane of the rack. The unitized disposable will have all the sample prep reagents, PCR reagents, process tubes as well as disposable pipettes already prepackaged in it. Once all disposables are loaded into the rack, the rack is placed in its location on the instrument. The user then places two 12-lane microfluidic PCR cartridges in the two trays of the PCR reader. The user then closes the door of the instrument and then starts the sample processing using the GUI (Graphical User Interface).

The instrument checks functionality of all subsystems and then reads the barcode of the sample tubes, the unitized reagent disposables and the microfluidic cartridges. Any mismatch with a pre-existing work list is determined and

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errors are flagged, if necessary. The instrument then goes through a series of liquid processing, heating, magnetic separation to complete the sample preparation steps for each of the clinical sample, mixes the purified nucleic acid with PCR reagents and dispenses the final mix into a lane of the microfluidic cartridges. After a microfluidic cartridge is loaded with the final PCR mix, the cartridge tray moves and aligns the cartridge in the reader and the optical detection system presses the cartridge against a microfluidic PCR heater surface. On-chip valves are actuated to close the reaction mix and then thermocycling is started to initiate the PCR reaction. At each cycle of PCR (up to 45 cycles), fluorescence from each PCR lane is detected by the optical detection system (2-colors per PCR lane) and final result is determined based on the threshold cycle (Ct).

The sample preparation steps for 24 samples are performed in about 40 minutes and the PCR reaction in about 20 minutes.

Sample Reader:

The Reader performs function testing of up to twelve properly prepared patient samples by PCR process (real-time PCR) when used in conjunction with HandyLab microfluidic (test) cartridges. Each unit will employ two Reader Modules for a total of up to twenty four tests. (FIGS. 72A and 72B) Operation of the Reader is designed for minimal customer interaction, requiring the loading and unloading of test cartridges only. During the "Load Disposables" sequence, the Reader will present a motor actuated tray for installation of the disposable cartridge. Sliding a small knob located in the front of the tray, a spring loaded protective cover will raise allowing the test cartridge to be nested properly in place. The cover is then lowered until the knob self-locks into the tray frame, securing the cartridge and preventing movement during the sample loading sequence.

Once the prepared samples have been dispensed via pipettes into the test cartridge, the tray will retract into the Reader, accurately positioning the test cartridge beneath the chassis of the optical assembly. The optical assembly will then be lowered by a captured screw driven stepper motor until contact is made with the test cartridge. At this point the test cartridge is located $\frac{1}{8}$ " above the target location on the heater assembly. As downward motion continues the test cartridge and its holder within the tray compress springs on the tray frame (these are used later to return the cartridge to its normal position and able to clear the encapsulated wire bonds located on the heater assembly during tray operation). Movement of the test cartridge and optical assembly is complete once contact with the heater assembly is made and a minimum of 2 psi is obtained across the two-thirds of the cartridge area about the PCR channels and their controlling gates. At this point the testing of the cartridge is performed using the heater assembly, measured with onboard optics, and controlled via software and electronics much in the same manner as currently operated on similar HandyLab instruments.

Once the functional testing is complete the main motor raises the optic assembly, releasing pressure on the test cartridge to return to its normal position. When commanded, the tray motor operating in a rack-and-pinion manner, presents the tray to the customer for cartridge removal and disposal. when the tray is in the extended position it is suspended above a support block located on the apparatus chassis. This block prevents the cartridge from sliding though the holder in the tray during loading and acts as a support while samples are pipetted into the disposable cartridge. Also provided in this support block is an assist lever to lift and grasp the disposable cartridge during

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removal. All components of the tray as well as support block and cartridge lift assist are removable by the customer, without tools, for cleaning and reinstalled easily. Microfluidic PCR Heater Module:

The microfluidic PCR heater module comprises a glass wafer with photolithographically defined microheaters and sensors to accurately provide heat for actuation of valves and performing thermocycling required to perform a real-time PCR reaction. The wafer surface has dedicated individually controlled heating zones for each of the PCR lanes in the microfluidic cartridge. For a 12-up cartridge, there are 12 PCR zones and the 24-up cartridge, there are 24 PCR heating zones. The individual heaters and sensors are electrically connected to a Printed circuit board using gold or aluminum wire bonds. A thermally compliant encapsulant provides physical protection the wirebonds. While the present device is made on glass wafer, heaters can be fabricated on Si-on-Glass wafers and other polymeric substrates. Each substrate can have provide specific advantages related to its thermal and mechanical properties. besides using photolithography process, such heating substrates can also be assembled using off-the-shelf electronic components such as power resistors, peltiers, transistors, maintaining the upper heating surface of each of the component to be at the same level to provide heating to a microfluidic cartridge. Temperature calibration values for each temperature sensor may be stored in a EEPROM or other memory devices co-located in the heater PCBoard.

12-Lane Cartridge:

This 12 channel cartridge is the same basic design that is described in U.S. provisional patent application Ser. No. 60/859,284, filed Nov. 14, 2006, with the following modifications: increase the PCR volume from 2 μ l to 4.5 μ l, leading to an increase in the input volume from 4 μ l to 6 μ l. The inlet holes are moved a few millimeters away from the edge of the cartridge to allow room for a 2 mm alignment ledge in the cartridge. A similar alignment ledge is also included on the other edge of the cartridge. (FIGS. 31A, 31B)

Enclosure:

The design of the apparatus enclosure must satisfy requirements: for customer safety during operation; provide access to power and communication interfaces; provide air entry, exit, and filtering; provide one-handed operation to open for installation and removal of materials; incorporate marketable aesthetics.

Cooling:

The cooling for the apparatus will be designed in conjunction with the enclosure and overall system to ensure all assemblies requiring air are within the flow path or receive diverted air.

The current concept is for the air inlet to be located on the bottom of the lower front panel. The air will then pass through a clearable filter before entering the apparatus. Sheet metal components will direct the air to both the disposable racks and the main power supply. The air will then be directed through the card cages, around the readers and will exit through slots provided in the top of the enclosure.

Base Plate

The XYZ stage and frame are mounted to the base plate in a way where there will be no misalignment between the stage, cartridge and the disposable. the enclosure is mounted to the base plate. Final design of the enclosure determines the bolt hole pattern for mounting. the backplane board mounts to the base plate with standoffs. All other boards mounts to the backplane board. The disposable mounts on a rack which will be removable from the brackets mounted to

the base plate. the reader brackets bolt to the base plate. Final design of the reader brackets determines the bolt hole pattern. The power supply mounts to the base plate. The base plate extends width and lengthwise under the entire instrument.

Example 12: Exemplary High-Efficiency Diagnostic Apparatus

A more highly multiplexed embodiment, also enables 24 clinical samples to be automatically processed to purify nucleic acids, mix the purified DNA/RNA with PCR reagents and perform real-time PCR in a microfluidic cartridge. This produce has a single PCR reader, with a scanning read-head, capable of reading up to 4 different colors from each of the PCR lane. The cartridge has 24 PCR channels enabling a single cartridge to run all 24 clinical samples. In addition, this product has a cartridge autoloader, whereby the instrument automatically feeds the PCR reader from a pack of cartridges into the instrument and discard used cartridge into a waste tray. Diagrams are shown in FIGS. 73, and 74.

The apparatus has the following sub-systems:

Two sample processing racks, each rack processes up to 12 clinical sample sin unitized disposable strips

Magnetic separator-cum-tube heater assembly (24 heating stations)

A four-probe liquid dispensing head

3-axis gantry to move the pipette head

A single PCR amplification-detection station capable of running a 24-lane microfluidic cartridge and a scanner unit to detect up to 4 colors from each PCR lane.

An autoloader unit to feed 24-lane microfluidic cartridges from a box into the PCR detection unit.

Control electronics

Barcode reader

Operation: The user will get a work list for each sample, whether they want to detect certain target analyte (such as GBS, Chlamydia, Gonorrhea, HSV) for each clinical sample. The sample tubes are placed on the rack and for each sample, the user slides in a unitized reagent disposable (analyte specific) into corresponding lane of the rack. The unitized disposable will have all the sample prep reagents, PCR reagents, process tubes as well as disposable pipettes already prepackaged in it. Once all disposables are loaded into the rack, the rack is placed in its location on the instrument. The user then closes the door of the instrument and then starts the sample processing using the GUI (Graphical User Interface).

The instrument checks functionality of all subsystems and then reads the barcode of the sample tubes, the unitized reagent disposables and presence of a 24-lane microfluidic cartridge. Any mismatch with a pre-existing work list is determined and errors are flagged, if necessary. The instrument then goes through a series of liquid processing, heating, magnetic separation to complete the sample preparation steps for each of the clinical sample, mixes the purified nucleic acid with PCR reagents and dispenses the final mix into a lane of a 24-lane microfluidic cartridge. After the microfluidic cartridge is loaded with the final PCR mix, the cartridge is moved and aligned by an automated motorized pusher in the PCR reader. The optical detection system, then presses the cartridge against a microfluidic PCR heater surface. On-chip valves are actuated to close the reaction mix and then thermo-cycling is started to initiate the PCR reaction. At each cycle of PCR (up to 45 cycles), fluorescence from each PCR lane is detected by the optical detec-

tion system (2-colors per PCR lane) and final result is determined based on the threshold cycle (Ct). The used cartridge is then pushed out automatically into a waste cartridge bin.

Microfluidic cartridges are stored in a cartridge pack (maximum 24 cartridges) and the instrument alerts the user to replace the cartridge pack and empty out the waste cartridge bin once all cartridges from the pack are used up. 24-Lane Cartridge

The 24-lane cartridge has two rows of 12 PCR lanes. Various views are shown in FIGS. 75-77. The cartridge has 3 layers, a laminate, a substrate, and a label. The label is shown in two pieces. Each Lane has a liquid inlet port, that interfaces with a disposable pipette; a 4 microliter PCR reaction chamber (1.5 mm wide, 300 microns deep and approximately 10 mm long), two microvalves on either side of the PCR reactor and outlet vent. Microvalves are normally open and close the channel on actuation. The outlet holes enables extra liquid (~1 µl) to be contained in the fluidic channel incase more than 6 µl of fluid is dispensed into the cartridge.

The inlet holes of the cartridge are made conical in shape and have a diameter of 3-6 mm at the top to ensure pipettes can be easily landed by the fluid dispensing head with the conical hole. Once the pipette lands within the cone, the conical shape guides the pipette and mechanically seals to provide error free dispensing or withdrawal of fluid into the cartridge. The bigger the holes, the better it is to align with the pipette, however, we need to maximize the number of inlet ports within the width of the cartridge as well as maintain the pitch between holes compatible with the inter-pipette distance. In this particular design, the inter-pipette distance is 18 mm and the distance between the loading holes in the cartridge is 8 mm. So lanes 1, 4, 7, 11 are pipetted into during one dispensing operation; lanes 2, 5, 8 and 12 in the next, and so on and so forth.

The height of the conical holes is kept lower than the height of the ledges in the cartridge to ensure the cartridges can be stacked on the ledges. The ledges on the two long edges of the cartridge enable stacking of the cartridges with minimal surface contact between two stacked cartridges and also help guide the cartridge into the reader from cartridge pack (cf. FIGS. 28-33).

Cartridge Autoloader

The Cartridge autoloader consists of a place for positively locking a pack of 24 microfluidic cartridges, pre-stacked in a spring-loaded box (e.g., FIG. 33). The box has structural elements on the sides to enable unidirectional positioning and locking of the box in the autoloader (FIG. 33). To load a new box, the user moves a sliding element to the left of the autoloader, places and pushed the box in the slot and released the sliding lock to retain the box in its right location. Springs loaded at the bottom of the box helps push the box up when it needs to be replaced. The spiral spring present at the bottom of the cartridge pack pushed against the cartridges and is able to continually push the cartridge with a force of from 4 to 20 pounds.

The presence or absence of cartridges is detected by reading the barcode on top of the cartridge, if present.

To start a PCR run, the pipette head dispenses PCR reaction mix into the required number of lanes in the top cartridge in the autoloader (e.g., FIG. 28). The pusher pushes the top cartridge from the autoloader box into the two rails that guide the cartridge into the PCR reader. The cartridge is pushed to the calibrated location under the reader and then the optics block is moved down using a stepper motor to push the cartridge against the microheater surface. the

bottom of the optics block (aperture plate) has projections on the sides to enable the cartridge to be accurately aligned against the apertures. The stepper motor pushed the cartridge to a pre-calibrated position (e.g., FIG. 30) which provides a minimum contact pressure of 1 psi on the heating surface of the microfluidic cartridge.

After the PCR reaction is complete, the stepper motor moves up 5-10 mm away from the cartridge, relieves the contact pressure and enables to cartridge to travel in its guide rails. The pusher is activated and it pushes the cartridge out to the cartridge waste bin (e.g., FIG. 32). After this step, the pusher travels back to its home position. During its back travel, the pusher is able to rise above the top of the cartridge in the cartridge pack because it has a angular degree of freedom (see figure). A torsion spring ensures the pusher comes back to a horizontal position to enable it to push against the next cartridge in queue. The pusher is mechanically attached to a timing belt. The timing belt can be moved in either direction by turning a geared motor. The pusher is mounted to a slider arrangement to constrain it to move in only one axis (see, e.g., FIG. 31).

The cartridge pushing mechanism can also be made to not only push the cartridge from the autoloader box to the detection position, but also be used to move it back to the autoloading position. This will enable unused lanes in the microfluidic cartridge to be used in the next PCR run.

The cartridge autoloading box is also designed so that once all the cartridges are used, the box can be easily recycled or new cartridges added to it. This reduces the cost to the customer and the manufacturer.

Reader

The reader consists of an optical detection unit that can be pressed against a 24-lane microfluidic cartridge to optically interface with the PCR lanes as well as press the cartridge against a microfluidic heater substrate (FIG. 78). The bottom of the optics block has 24 apertures (two rows of 12 apertures) that is similar in dimension of the PCR reactors closest to the cartridge. The aperture plate is made of low fluorescent material, such as anodized black aluminum and during operation, minimized the total background fluorescence while maximizing the collection of fluorescent only from the PCR reactor (FIGS. 79A and 79B). The bottom of the aperture plate has two beveled edges that help align two edges of the cartridges appropriately such that the apertures line up with the PCR reactors. (FIGS. 80, 81)

The optical detection units (total of 8 detection unit) are assembled and mounted onto a sliding rail inside the optical box so that the optical units can be scanned over the apertures (FIG. 82). Each unit is able to excite and focus a certain wavelength of light onto the PCR reactor and collect emitted fluorescence of particular wavelength into a photo-detector. By using 4 different colors on the top 4 channels and repeating the 4 colors in the bottom channels, the entire scanner can scan up to 4 colors from each of the PCR lanes.

The optics block can be machined out of aluminum and anodized or injection molded using low fluorescence black plastic (FIG. 83). Injection molding can dramatically reduce the cost per unit and also make the assembly of optics easier. The designed units can be stacked back-to-back.

Example 13: Exemplary Electronics for Use with Preparatory and Diagnostic Apparatuses as Described Herein

There are multiple independent software modules running on dedicated hardware: Described herein are exemplary specifications for the electronics used in the diagnostic

(PCR) system. Additional information related to the PCR System is described elsewhere herein. In some embodiments, the PCR system includes eighteen printed circuit boards (PCBs) of nine different types. Referring to FIG. 86, the system can contain three multiplex (MUX) boards 100a-c, two of which (micro-heater MUX boards 100a-b), can each be used to run a micro-heater board 110a-b and the third (lysis heater MUX board 100c) can run one or more lysis heater boards 116 and 117. Each of the three MUX boards 100a-c can be controlled by a PC processor board via an Ethernet port. The two micro-heater boards 110a-b, each controlled by one of the MUX boards 100a-b, heat micro-zones on the microfluidic cartridge. In some embodiments, the system includes the two lysis heater boards 116 and 117, controlled by the lysis heater MUX board 100c, that heat lysis tubes in each of the two 12 samples racks.

Still referring to the PCBs included in the PCR system, the system can include two 12-channel optical detection boards 130a-b that can each detect optical fluorescence emitted by microfluidic cartridge chemistry. The optical detection boards can be controlled by one or more of the MUX boards 100a-c, using SPI, over a RS-422 interface. The system can include three motor control board 140a-c, where one board (e.g., motor control boards 140c) can control two magnetic separation motors (not shown), and the remaining two motor control boards (e.g., motor control boards 140a-b) can each run one reader tray motor (not shown) and one reader pressure motor (not shown). The motor control board running the magnetic separation motors (e.g., motor control board 140c) can be controlled via RS-485 interface from the lysis heater MUX board 100c and the two motor control boards 140a-b, each running one reader tray motor and one reader pressure motor, can be controlled via RS-485 interface by the micro-heater MUX boards 100a-b. The system can also include one PC processor boards 150, which directs the overall sequencing of the system and can be controlled via external Ethernet and USB interfaces, and one PC processor base board 160, which provides internal interfaces for the PC processor board 150 to the remainder of the system and external interfaces. The system can include one main backplane 180 that interconnects all system boards, one motor control backplane 190 that interconnects the motor control boards 140a-c to the main backplane 180 and gantry (not shown), and two door sensor boards (not shown). One door sensor board provides an interconnect between the front door solenoid locks and the PC processor base board 160 and the other door sensor board provides an interconnect between the position sensors and the PC processor base board 160.

In some embodiments, the PCR system can include the off-the-shelf PC processor board 150. The PC processor board 150 can be an ETX form factor board that includes one 10/100 BASE-T Ethernet port, four USB ports, one analog VGA display port, two UART ports, one real-time clock, one parallel port, one PS2 keyboard port, and PS2 mouse port, stereo audio output, one IDE interface, and one 12C interface.

Referring to FIG. 87, the system can also include the PC processor base board 160 that includes a five port 10/100 BASE-T Ethernet bridge 161 for internal communication, one of which can be connected to the 10/100 BASE-T Ethernet port of the PC Processor board 150, another of which can be for diagnostic use (with a connector inside system cover), and three of which can communicate with the three MUX boards 100a-c (one port for each MUX board 100a-c) through the backplane 180. The PC processor base board 160 can also include on USB to 10/100 BASE-T

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Ethernet port **162** for external Ethernet connections, one four port USB hub **163** for external connections, one external VGA connector **164**, one internal PS2 Mouse connector **165** (with a connector inside the system cover), and one internal PS2 Keyboard connector **166** (with a connector inside the system cover). The PC processor base board **160** can also include one internal stereo audio output **167** to on board speakers **168**, one internal CompactFlash connector **169** from an IDE port (with a connector inside the system cover), and one internal RS-232 interface **170** from a UART port (with a connector inside the system cover). Additional components included in the PC processor base board can include one internal RS-485 interface **171** from a UAT port (with a connector inside the system cover), one internal temperature sensor **172** connected to the 12C interface, a battery for the real-time clock, and one parallel port **173**. The parallel port **173**, with connectors inside the system cover, can be internally connected as follows: one bit can be used to drive a high current low side switch for the two door solenoids, one bit can be used to generate a processor interrupt when either door sensor indicates that a door is opened, three bits can be used to program the EEPROM for configuring the Ethernet bridge **161**, and two bits can be connected to the Ethernet bridge management interface (not shown). The remaining bits can remain unassigned, with optional pull-up and pull-down resistors, and be brought out to a 10 pin Phoenix contact header.

Referring now to FIG. **88**, in some embodiments, the system can include the three MUX boards **100a-c**. While FIG. **88** depicts exemplary MUX board **100a**, each of the three MUX boards **100a-c** can include one or more of the features described below. The MUX board **100a** can include 96 pulse width modulated (PWM) controlled heating channels with heaters (about 33 ohm to about 150 ohm) heaters, that can support 20 or 24 volt (voltage externally provided) drives with a maximum current of about 800 mA. Each PWMs can be 12-bit with programmable start and stop points, can have 1 microsecond resolution, and can have a maximum duty cycle of about 75%. Each PWM period is programmable and is preferably set to 4 ms. The MUX boards can include a 4-wire RTD/heater connection with precision 1 mA sense current that can accommodate about 50 ohm to about 2500 ohm resistive temperature devices and have a measurement accuracy of ± 0.5 ohms. The thermal measurement sample period of the MUX boards is 32 ms including 8x PWM periods where 12 16-bit ADCs **101a** sample 8 successive channels each. The MUX address can be tagged to the ADC data.

Still referring to the MUX board **100a** depicted in FIG. **88**, as RS-422 optics board interface **102a** that interconnects over the backplane **180** and transfers data over a 4 wire SPI interface using local handshake signals and interrupts can be included on the MUX board **100a**. The MUX board **100a** can also include a 10/100 BASE-T Ethernet interface **103a** that interconnects to the system over the backplane **180** and an RS-485 interface **104a** that interconnects to the motor controller **140a** over the backplane **180**.

Referring now to FIG. **89**, in some embodiments, the system can include the optical detection boards **130a-b**. While FIG. **89** depicts exemplary optical detection board **130a**, each of the optical detection boards **130a-b** can include one or more of the features described below. The optical detection board **130a** can include a 12-channel optics board design modified to use an RS-422 interface **131a**. The optical detection board **130a** can include 12—3 Watt, blue LEDs **132a** drives with about 6 V at about a 625 mA maximum. An exemplary LED used in the detection board

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130a is the Luxeon K2 emitter producing blue light at a wavelength of about 470 nm using about 27 mW @ 700 mA. The optical detection board **130a** can also include 12—3 Watt, amber LEDs **133a** driven with about 6 V at about a 625 mA maximum. An exemplary LED used in the detection board **130a** in the Luxeon K2 emitter producing amber light at a wavelength of about 590 nm using about 60 mW @ 700 mA. The detection board **130a** can include 24 lensed silicon photodiode detectors **134a**, an example of which is the Hamamatsu S2386-18L. These photodiode detectors **134a** are designed in a common TO-18 package. The detection board **130a** can also include an MSP430 processor **135a** with two PWM channels, one for the blue channel and one for the amber channel. The board **130a** can include individual LED enables **136a** and **137a** for each of the 12 color pairs set over the local SPI bus.

The PCR system can include a lysis heater board that provides and monitors heating to the lysis tubes. The heater board can include 12—70 Watt TO-247 power resistors (provide heat to the lysis tubes) designed to be fed 24V from one or more of the MUX boards **100a-c** (e.g., MUX board **100c**) and 12—2000 ohm Resistive Temperature Devices (RTD) to monitor the temperature of the lysis tubes. Optional resistors can be included to modify the full scale range of the RTDs. Included on the lysis heater board is a serial EEPROM that may hold a board serial number and can be used to identify the board type and revision level to software.

Referring now to FIG. **90**, in some embodiments, the system can include the micro-heater boards **110a-b**. While FIG. **90** depicts exemplary micro-heater board **110a**, each of the micro-heater boards **110a-b** can include one or more of the features described below. In some embodiments, the system can include the micro-heater board **110a** that includes a serial EEPROM and two optical interrupts. The serial EEPROM may hold a board serial number, can hold RTD calibration data, and can be used to identify the board type and revision level to software. The optical interrupters can be used to sense the reader tray position for the motor control board **140a** and sends the information to the Blue Cobra (motor controllers), which processes the information on the positions of the reader trays and accordingly controls the power to the emitters supplied by the motor control board **140a**. The micro-heater board **110a** can provide connections to the 96 channel micro-heater plate and control the 96 multiplexed heater/RTD devices to control cartridge feature temperature. The heater/RTD devices can be between about 50 ohms to about 500 ohms. The micro-heater board **110a** can bridge the RS-422 interface from, for example, the MUX board **100a** to the optical detection board **130a**. The connection from the micro-heater board **110a** to the MUX board **110a** is over the backplane **180**, while the connection to the optics board **130a** is over a 40 pin FFC cable.

Referring now to FIG. **91**, in some embodiments, the system can include the motor control boards **140a-c**. While FIG. **91** depicts exemplary motor control board **140a**, each of the motor control boards **140a-c** can include one or more of the features described below. In some embodiments, the system can include the motor control board **140a** that can control two micro-stepping motors **141a** and can be connected to the backplane **180** via a RS-485 interface. The output to the motors can be up to 24 V supplied externally through the backplane **180**. The output current can be jumper selectable. Exemplary output currents that can be selected via jumper settings can include about 700 mA, about 1.0 A, or 2.3 A. The motor control board **140a** includes

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open collector TTL interrupt output to the MUX board **100a** and flag inputs. The flag inputs can provide 1.5 V power output to the sensors and can be switched on and off by software.

Limit switches are placed on the extreme locations of each axis, e.g., x-minimum and x-maximum, that turns off the power to the motor driving that axis in case of a malfunction happens and the pipette head moves out of the designed working distance. Optional pull-up and pull-down are used with the output of the optical interrupters.)

In some embodiments, the system can include one or more interconnection boards, such as the main backplane **180**. The main backplane **180** can interconnect other PCBs, such as the MUX boards **100a-c**, PC processor base board **160**, and heater Interconnect boards. The main backplane **180** can cable to the motor control backplane **190** and to two lysis heater boards. The main backplane **180** can distribute power and signaling, implement 10/100 BASE-T Ethernet and RS-485 over the backplane **180**, and supplies voltages from an external connector. Exemplary voltages supplied include +3.3 V, +5.0 V, +12.0 V, -12.0 V, +20.0 V, and +24.0 V.

The system can include the motor control backplane **190** that can distribute power and signaling for all of the motor control boards **140a-c**. The motor control backplane **190** can supply +5.0 V and 24.0 V from an external connector. The motor control backplane **190** can include 1 slot for the RS-485 signaling from each of the two MUX boards **100a-b** (total of 2 slots), 6 slots for the RS-485 signaling from the lysis heater controlling MUX board **100c**, and one connector that provides RS-485 signaling and power to the gantry. The motor control backplane **190** can provide pull-up and pull-down resistors to handle floating buses.

In some embodiments, the system can include a heater interconnect board and a door sensor board. The heater interconnect board can connect the micro-heater boards **110a-b** to the main backplane **180** using a physical interconnect only (e.g., no active circuits). the door sensor board can provide a cable interface and mixing logic fro the optical interrupters, which sense the door is open, and provide a mounting and cabling interface to the door lock solenoid.

Example 14: Exemplary Software for Use with Preparatory and Diagnostic Apparatuses as Described Herein

There are multiple independent software modules running on dedicated hardware:

- Reader (2);
- Sample-Prep (1);
- User Interface (1);
- Detector (2);
- Motor control (8)

Inter-module communication among is via an internal Ethernet bus, communication with the user interface is via a high speed SPI bus and communication with motor control via a RS485 serial bus.

The Reader and Sample-Prep software run on identical hardware and are as such identical incorporating the following functions:

- Script Engine (a parametrized form of a protocol)
- Protocol Engine
- Temperature Control (Microfluidics, lysis, release)
- Motor control (via external motor control modules).
- Salient features of the motor control software are:
 - Command/reply in ASCII and addressing capability to allow daisy chaining of communication link.

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Detection (via external detector modules) Detector module controls the LED illumination and photo detector digitization.

The user interface is implemented as a program running under Linux operating system on an embedded x86 compatible PC. The following functions are addressed:

- Graphical User Interface
- Test control and monitor
- Test result storage and retrieval Network connectivity via Ethernet (to lab information systems)
- USB interface
- Printer
- Scanner (Internal and external)
- Keyboard
- Mouse
- Door lock and sense

Example 15: Exemplary Chemistry and Processes of Use

Chemistry Overview:

The chemistry process centers around the detection and identification of organisms in a clinical specimen, by virtue of detecting nucleic acids from the organism in question. This involves isolation of nucleic acids from target organisms that are contained in a clinical specimen, followed by a process that will detect the presence of specific nucleic acid sequences. In addition to target detection, an internal positive control nucleic acid will be added to the collection buffer, and will e taken through the entire extraction and detection process along with target nucleic acids. This control will monitor the effectiveness of the entire process and will minimize the risk of having false negative results.

Nucleic Acid Extraction and Purification:

Nucleic acid extraction procedures begin with the addition of a clinical specimen to a prepared specimen collection solution. This can be done either at a specimen collection site, or at the testing site. Two collection solution formats will be available: one for body fluids, and one for swab specimens. Collection solutions used at collection sites will serve as specimen transport solutions, and therefore, this solution must maintain specimen and analyte integrity.

The extraction and purification procedure, which is entirely automated, proceeds as follows:

- Target organisms are lysed by heating the detergent-containing collection solution.

- Magnetic beads, added to the specimen/collection solution mix, non-specifically bind all DNA that is released into the solution.

- Magnetic beads are isolated and are washed to eliminate contaminants

- DNA is released from the beads using high pH and heat.

- DNA containing solution is removed and neutralized with a buffer

Nucleic Acid Amplification:

Nucleic acids that have been captured by magnetic beads, washed, released in high pH, and neutralized with buffer, are added to a mixture of buffers, salts, and enzymes that have been lyophilized in a tube. The mixture is rapidly rehydrated, and then a portion of the solution is loaded onto a microfluidic cartridge. The cartridge is then loaded into the amplification instrument module, which consists of a heat-

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ing unit capable of thermal cycling, and an optical detection system. Detection of target nucleic acids proceeds as follows:

The liquid is sealed in a reaction chamber.

Rapid thermal cycling is used to potentiate the Polymerase Chain Reaction (PCR), which is used to amplify specific target DNA.

Amplified DNA fluoresces, and can be detected by optical sensors.

A fluorescent probe "tail" is incorporated into each amplified piece of DNA

At a specific temperature, the probe adopts a conformation that produces fluorescence (this is termed a "scorpion" reaction, see FIG. 84).

Fluorescence is detected and monitored throughout the reaction.

Extraction and Amplification/Detection Process:

Extensive bench-scale testing has been performed to optimize the nucleic acid extraction chemistry, including the collection buffer, the wash buffer formulation, the release solution formulation, and the PCR reagent mixes. The fully automated method of extraction, followed by 12-up PCR, was able to provide very high sensitivity consistently at 150 copies/sample.

Examples: Chlamydia in Urine (50/50); Gonorrhea in Urine; GBS in Plasma.

Various detection chemistries such as Taqman, Scorpion, SYBRg Green work reliably in the microfluidic cartridge. Reagent Manufacturing

Feasibility studies were conducted in order to determine whether PCR reagents could be lyophilized in PCR tubes besides the use of 2 µl lyophilized pellets. The studies have indicated that sensitivity of reactions performed using tube-lyophilized reagents is equivalent to that of wet reagents or 2 µl pellet reagents, so feasibility has been proven. Stability studies for this format indicate similar stability data. We have seen 2 microliter lyophilized PCR pellets to be stable to up to 2 years at room temperature, once sealed in nitrogen atmosphere.

Manufacturing Overview: Manufacturing the components of the system can be accomplished at HandyLab, Inc., Ann Arbor, Mich. The manufacturing task has been split into five areas that consist of: chemistry manufacture, disposable strip, collection kit, cartridge and analyzer.

Chemistry Manufacturing: There are currently seven individual, blended chemistry components identified for potential use with the system described herein. Mixing, blending and processing reagents/chemicals can be performed at HandyLab, Inc., with existing equipment already in place. Additional tooling and fixtures will be necessary as the product matures and we ramp to high volume production, but initial costs will be minimal.

Collection buffer, wash, release & neutralization liquids are simple recipes with very low risk, and can be made in large batches to keep labor costs of mixing/blending at or below targeted projections. They will be mixed and placed into intermediate containers for stock, and then issued to Disposable Strip Manufacturing for dispensing. Mature SOP's are in place from prior project activity.

Affinity Beads (AB) have good potential to be stored and used as a liquid in the strip, but design contingencies for using a lyophilized pellet are in place as a back up. It is critical to keep the beads suspended in solution during dispense. Dispense equipment (e.g., manufactured by Innovadyne) that provides agitation for continuous suspension during dispense has been identified for purchases once stability has been proven for liquid AB storage in the strip.

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The process to manufacture and magnetize the Affinity Beads spans a 9 hour cycle time to produce a batch of 2,000 aliquots, but that same time period can be used for sealed up recipe batches once we ramp into high volume production. This item has the highest labor content of all chemistry manufacture that is currently required for the apparatus.

PCR reagents/enzymes will be freeze-dried in our existing lyophilizing chamber (Virtis Genesis) but will not require spherical pellet formation. Instead, the mixture is being dispensed into, and then lyophilized, inside the end-use tube. First the chemistries are mixed per established SOPs, and then the following steps are performed to accomplish lyophilization: Individual tubes are placed into a rack/fixture, and the solution is dispensed into each, using existing equipment (EFD Ultra Dispense Station.) The filled rack will be placed inside a stainless steel airtight box (modified to accept stoppers in the lid,) and then placed into the lyophilization chamber and the drying cycle commences unattended. During lyophilization, the stoppers are in a raised position allowing air/nitrogen to circulate into, and moisture to exit the stainless box holding racks of vials. At the end of the cycle, the shelves of our lyophilization chamber lower to seat the stoppers into the lid, forming a seal while still inside the closed chamber, in a moisture free nitrogen atmosphere. The steel boxes are then removed from the chamber, and each rack inside shall be processed in a single operation to seal all vials in that rack. Immediately after sealing, the vials will be die cut from the full in one operation, allowing individual vials to be forwarded to the Disposable Manufacturing area for placement into a strip. Internal Control will either be added to an existing solution, or will be dispensed into its own cavity in the manner of the collection buffer, wash, neutralization, and release solutions. If lyophilization is required, it will be accomplished in the same manner as the PCR chemistry, and later snapped into the strip. Shelf life stability studies are underway.

Collection Kit Manufacturing

The collection kit will be processed manually in house for initial quantities. Initial quantities will not require capital expenditures as we have all equipment necessary to enable use to meet projections through 2008. We will be using our existing equipment (EFD 754-SS Aseptic Valve & Valve-mate 7000 Digital Controller,) to fill the collection vial. The vials have a twist-on top that will be torqued, and the vial will have a proprietary ID barcode on each vial. 24 vials will be placed into a reclosable plastic bag and placed into a carton for shipping.

Place vials into rack.

Dispense solution into vials.

Install and torque caps.

Label vials.

Bag vials and label bag.

Place vial bag and instructions/insert into carton, close and label.

Cartridge Manufacturing:

Existing semi-automatic equipment for laminating & waxing (Think & Tinker DF-4200, & Asymtek Axiom Heated Jet Platform, respectively,) will be utilized to meet all cartridge manufacture requirements. The footprint of the 12-up disposable is the same as the RTa10 cartridge, so additional fixtures are not necessary.

Laminate micro substrate & trim excess.

Fill valves with hot wax & inspect.

Apply label & barcode.

Band 24 pieces together.

Bag & seal banded cartridges, label bag.

Place bag & insert(s) into carton, seal and label.

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This portion of the product is relatively simple, although there is a difference between the automated (as used herein) and the stand-alone 12-up cartridge. Venting will not be required on the cartridge, which eliminates the most time consuming process for cartridge manufacture, along with the highest risk and highest cost for fully integrated automation. Over 1,000 pieces of the 12-up with venting have been successfully produced.

Example 16: Exemplary Chemistry Processes

Sample Pre-Processing

For Urine Sample: Take 0.5 ml of urine and mix it with 0.5 ml of HandyLab collection buffer. Filter the sample through HandyLab Inc.'s pre-filter (contains two membranes of 10 micron and 3 micron pore size). Place the sample tube in the position specified for the external sample tube in the 12-up rack.

For Plasma Sample: Take 0.5 ml of plasma and mix it with 0.5 ml of HandyLab collection buffer. Place the sample tube in the position specified for the external sample tube in the 12-up rack.

For GBS swab samples: Take the swab sample and dip it in 1 ml of HandyLab collection buffer. Place the sample tube in the position specified for the external sample tube in the 12-up rack.

The HandyLab sample collection buffer contains 50 mM Tris pH 7, 1% Triton X-100, 20 mM Citrate, 20 mM borate, 100 mM EDTA, plus 1000 copies of positive control DNA.

Loading the Instrument and Starting Sample Processing

1. Load PCR tube containing PCR master mix in one of the specified snap-in location of the unitized disposable.
2. Load PCR tube containing PCR probe and primers for the target analyte under consideration in the specified location of the unitized disposable.
3. In case of two analyte test, local PCR tube containing probes and primers for second analyte in the specified location of the unitized disposable.
4. Load the unitized disposable in the 12-up rack in the same lane as the sample tube under consideration.
5. Prepare and load unitized reagent strips for other samples in consideration.
6. Load the 12-up rack in one of the locations in the instrument.
7. Load 12-up cartridge in the cartridge tray loading position.
8. Start operation.

Liquid Processing Steps

1. Using Pipette tip #1, the robot transfers the clinical sample from the external sample tube to the lysis tube of the unitized disposable strip.
2. Using the same pipette tip, the robot takes about 100 μ l of sample, mixes that lyophilized enzyme and affinity beads, transfers the reagents to the lysis tube. Mixing is performed in the lysis tube by 5 suck and dispense operations.
3. The robot places pipette tip #1 as its designated location in the unitized disposable strip.
4. Heat the lysis tube to 60 C and maintain if for 10 minutes.
5. After 5 minute of lysis, the robot picks up pipette tip #1 and mixes the contents by 3 suck and dispense operations.
6. The robot places pipette tip #1 at its designated location in the unitized disposable strip.

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7. After 10 minutes of lysis, a magnet is moved up the side of the lysis tube to a middle height of the sample and held at that position for a minute to capture all the magnetic beads against the wall the tube.
8. the magnet is brought down slowly to slide the captured beads close to the bottom (but not the bottom) of the tube.
9. Using pipette tip #2, aspirate all the liquid and dump it into the waste tube.
10. Aspirate a second time to remove as much liquid as possible from the lysis tube.
11. Using the same pipette tip #2, withdraw 100 μ l of wash buffer and dispense it in the lysis tube. During this dispense, the magnet is moved downwards, away from the lysis tube.
12. Perform 15 mix steps to thoroughly mix the magnetic beads with the wash buffer.
13. Wait for 30 seconds.
14. Move magnet up to capture the beads to the side and hold for 15 seconds.
15. Using pipette tip #2, aspirate wash buffer twice to remove as much liquid as possible and dump it back in the wash tube.
16. Move magnet down away from the lysis tube.
17. Place pipette tip #2 in its specified location of the unitized disposable strip.
18. Pick up a new pipette tip (tip #3) and withdraw 8-10 μ l of release buffer and dispense it over the beads in the lysis tube.
19. Wait for 1 minute and then perform 45 mixes.
20. heat the release solution to 15° C. and maintain temperature for 5 minutes.
21. Place pipette tip #3 in its specified location of the unitized disposable strip.
22. Bring magnet up the tube, capture all the beads against the tube wall and move it up and away from the bottom of the tube.
23. Pick up a new pipette tip (tip #4) and withdraw all the release buffer from the lysis tube and then withdraw 3-10 μ l of neutralization buffer, mix it in the pipette tip and dispense it in the PCR tube. (In case of two analyte detections, dispense half of the neutralized DNA solution into first PCR tube and the rest of the solution in the second PCR tube.
24. Using pipette tip #4, mix the neutralized DNA with the lyophilized reagents by 4-5 such and dispense operations and withdraw the entire solution in the pipette tip.
25. Using pipette tip #4, load 6 μ l of the final PCR solution in a lane of the 12-up cartridge.

The usage of pipette heads during various processes is shown schematically in FIGS. 85A-C.

Real-Time PCR

After all the appropriate PCR lanes of the PCR cartridge is loaded with final PCR solution, the tray containing the cartridge moves it in the PCR Analyzer. The Cartridge is pressed by the Optical detection read-head against the PCR heater. Heaters activate valves to close either ends of the PCR reactor and real-time thermocycling process starts. After completing appropriate PCR cycles (~45 cycles), the analyzer make a call whether the sample has the target DNA based on the output fluorescence data.

Pipette Detection

The pipette head has 4 infrared sensor for detecting the presence of pipettes. This is essential to ensure the computer positively knows that a pipette is present or missing. Since pipettes are picked up using mechanical forcing against the

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pipette and also dispensed using mechanical motion of a stripper plate, pipette sensing helps preventing errors that otherwise may happen.

Force Sensing of the Pipette Head

The multi-pipette head is assembled in such a way and a force sensor interfaced with it so that any time the pipette head seats against the disposable pipette(s) or the picked pipettes are forced through the laminate in the reagent disposable or the pipette is forced against the bottom of the tubes in the reagent disposable, an upward force acts on the pipette head through the pipette holding nozzle or the pipettes itself. The entire head is pivoted, as shown in Figure and any force acting on the head causes a set-screw on the upper part of the head to press against a force sensor. This force sensor is calibrated for vertical displacement of the head against a non-moving surface. Using this calibration, it can be determined when to stop moving the head in the z-direction to detect whether pipettes are properly seated or if pipettes hit tube bottoms.

Alignment of Pipette Tips While Loading PCR Reagents Into the Microfluidic Cartridge

The pipettes used in the apparatus can have volumes as small as 10 μ l to as large as 1 ml. Larger volume pipettes can be as long as 95 mm (p1000 pipette). When 4 long pipette tips are sprung from the head, even a 1° misalignment during seating can cause the tip to be off-center by 1.7 mm. As it is impossible to have perfect alignment of the tip both at the top where it is interfaced with the tip holder and the bottom, it becomes necessary to mechanically constrain all the tips at another location closer to the bottom. We have used the stripper plate, having a defined hole structure to use it to align all the tips. The stripper plate hole clears all the 4 pipette tips when they are picked up. After the tips are properly seated, the stripper plate is moved in the x-axis using a motor to move all the pipettes against the notch provided in the stripper plate (see FIG. 46b). Now all the pipettes land on the cartridge inlet holes with ease.

Sample Preparation Extensions

The current technology described details of processing clinical samples to extract polynucleotides (DNA/RNA). The same product platform can be extended to process samples to extract proteins and other macromolecules by changing the affinity molecules present in the magnetic beads. The amplification-detection platform can also be used to perform other enzymatic reactions, such as immunoPCR, Reverse-transcriptase PCR, TMA, SDA, NASBA, LAMP, LCR, sequencing reactions etc. The sample preparation can also be used to prepare samples for highly multiplexed microarray detections as well.

Example 16: Exemplary Material for RNA-Affinity Matrix

An exemplary polynucleotide capture material preferentially retains polynucleotides such as RNA on its surface when placed in contact with a liquid medium that contains polynucleotides mixed with other species such as proteins and peptides that might inhibit subsequent detection or amplification of the polynucleotides.

The exemplary polynucleotide capture material is: Polyamidoamine (PAMAM) Generation 0, available from the Sigma-Aldrich Chemical Company ("Sigma-Aldrich"), produce number 412368. PAMAM is a dendrimer whose molecules contain a mixture of primary and tertiary amine groups. PAMAM (Generation 0) has the structure shown herein.

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The PAMAM, during use, is immobilized on a solid support such as carboxylated beads, or magnetic beads. The polynucleotide capture material comprises polycationic molecules during an operation of polynucleotide capture. Affinity between the material and polynucleotides is high because polynucleotides such as DNA and RNA typically comprise polyanions in solution.

After polynucleotide molecules are captured on a surface of the material, and remaining inhibitors and other compounds in solution have been flushed away with an alkaline buffer solution, such as sequence 0.1 mM Tris (pH 8.0), the polynucleotides may themselves be released from the surface of the material by, for example, washing the material with a second, more alkaline, buffer, such as Tris having a pH of 9.0.

Exemplary protocols for using PAMAM in nucleic acid testing are found in U.S. patent application Ser. No. 12/172,214 filed Jul. 11, 2008, incorporated herein by reference.

Example 17: Exemplary Material for DNA-Affinity Matrix

The exemplary polynucleotide capture material is: Polyethyleneimine (PEI), available from the Sigma-Aldrich Chemical Company ("Sigma-Aldrich"), product number 408719.

Exemplary protocols for using PEI in nucleic acid testing are found in U.S. patent application Ser. No. 12/172,208 filed Jul. 11, 2008, incorporated herein by reference.

Example 18: Exemplary Apparatus

Described herein are exemplary specifications for the mechanical design of the PCR system. In some embodiments, the system can be about 28.5 inches deep, or less, and about 43 inches wide, or less, and weight about 250 pounds or less. The system can be designed with a useful life of about 5 years (e.g., assuming 16,000 tests per year) and can be designed such that the sound level for this instrument (during operation) does not exceed 50 dB as measured 12 inches from the instrument in all ordinate directions. In some embodiments, the exterior of the system can be white with texture.

Referring to the overall system, in some embodiments, critical components of the system can remain orthogonal or parallel (as appropriate) to within 0.04 degrees. Exemplary critical components can include motion rails, pipettes, nozzles (e.g., axially as individual nozzles, linearly as an array of four nozzle centroids, or the like), lysis heaters, major edges of the installed cartridge holder in the reader drawer, the front face of the separation magnets, and the like. In the following descriptions, the X-axis (or X direction) refers to the axis extending from left to right when facing the front of the system, the Y-axis (or Y direction) refers to the axis extending from back to front when facing the front of the system, and the Z-axis (or Z direction) refers to the axis extending up from the bottom when facing the front of the system. As viewed from the top of the instrument, the centroid of the leftmost pipette nozzle on the Z-payload (as viewed from the front of the instrument) can be capable of unobstructed travel in the X direction from a point 80 mm from the outermost left baseplate edge to a point 608 mm from the outermost left baseplate edge and can be capable of unobstructed travel in the Y direction from a point 60 mm from the outermost front baseplate edge to a point 410 mm from the outermost front baseplate edge.

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Still referring to the system, as viewed from the front of the instrument, the bottom-most face of the pipette nozzles on the Z-payload can be capable of unobstructed travel in the Y direction from a point 156 mm above the top surface of the baseplate to a point 256 mm above the top surface of the baseplate. The 1 ml pipette tips can be capable of penetrating the foil covers included on disposable reagent strips. This penetration may not create contamination, affect the associated chemistries, or damage the pipette tips. Motions can be executed in such a manner as to eliminate mechanical hysteresis, as needed. Gantry motions can be optimized to prevent cross lane contamination and carryover. The rack can align the reagent strips to a tolerance of ± 0.010 inches in the X and Y directions.

Referring now to the gantry, in some embodiments, the gantry can consist of a stepper-motor actuated, belt/screw-driven cartesian robotic system. The gantry can be free to move, with or without attachments, above the modules that are forward of the rear facade and below the bottom-most horizontal face on the Z head, so long as the Z-payload is fully retracted. The gantry can be capable of travel speeds up to about 500 mm/sec in the X and Y directions and up to about 100 mm/sec in the Z direction. The accuracy and precision of the axis motions (e.g., with respect to the X, Y, and Z home sensors) can be 25 mm or better for each axis, and can be retained throughout the maintenance period. The axis drive belts may not leave residue in areas where PCR and samples are processed. The gantry can contain provisions for routing its own and all Z-payload wire harnesses back to the instrument. Belt tension on the X and Y axes can be set at 41.5 \pm 3.5 pounds.

Referring now to the Z-payload, the fluid head can have 4 pipette attachment nozzles located on 24 mm centers. Exemplary pipette tips that the pipette nozzles can capture without leakage include Biorobotix tips PN23500048 (50 μ L), PN23500049 (1.75 μ L), and PN23500046 (1 ml). The Z payload can incorporate a stepper actuated stripper plate capable of removing pipetted tips (e.g., the pipette tips described above). The system can include a pump and manifold system that includes software controlled aspiration, dispensing, and venting of individual fluid volumes within each of the four individual tips and simultaneous dispensing and venting on all tips. The pump and manifold system can have an accuracy and precision of about ± 2 μ L per tip for volumes that are less than 20 μ L and about $\pm 10\%$ for volumes greater than or equal to 20 μ L (e.g., when aspirating or dispensing in individual tips). The total pump stroke volume can be greater than about 8 μ L and less than about 1250 μ L. The minimum aspirate and dispense speed can be about 10 μ L/sec to about 300 μ L/sec. The centroid of the bottom-most face of each pipette tip can be axially aligned with the nozzle centroid of the pipette nozzles within 0.2 mm. The bottom-most pipette tip faces can be co-planar within 0.2 mm. The Z-payload can incorporate a Z axis force sensor capable of feedback to software for applied forces of between about 0 and 4 lbs. The Z-payload can incorporate a downward facing barcode reader capable of reading the system barcode as described elsewhere herein.

Referring now to racks included in the system, disposable reagent strips (e.g., oriented orthogonally to the front of the instrument) can be contained in 2, 12-lane racks. The 12 reagent strips in a given rack can register and lock into the rack upon insertion by a user. The rack can contain an area for 12 sample lysis tubes (e.g., PN 23500043) and hold the tube bottoms co-planar, allowing the user to orient the barcode to face the rear of the instrument. Certain features,

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including those listed above, can allow the racks to be inserted and oriented in the instrument by a minimally trained user. Proper rack placement can be confirmed by feedback to the software. In some embodiments, the racks can be black and color first (e.g., the color may not appreciably degrade with use or washing with a 10% bleach solution) and the rack material can be dimensionally stable within 0.1 mm over the operating temperature range of the system. The rack can be designed with provisions to allow the rack can be carried to and from the instrument and to minimize or eliminate the likelihood that the tubes held by the rack will spill when placed on a flat surface.

Referring now to the reader and PCR heater included in the system, the reader can allow for cartridge insertion and removal by, for example, a minimally trained user. The cartridge can remain seated in the reader during system operation. In some embodiments, the cartridge barcode may not be read properly by the barcode scanner if the cartridge is inserted incorrectly (e.g., upside down or backwards), thus the system can instruct a user to correctly reinsert the cartridge into the reader tray when the cartridge is inserted incorrectly. The reader drawer can repeatedly locate the cartridge, for loading by the pipette tips, within 0.5 mm. The reader can deliver the cartridge from the loading position into a react and detect position by means of an automated drawer mechanism under software control. The PCR lanes of the cartridge can be aligned, with both the optical system and heater, by the reader tray and drawer mechanism. The cartridge can contact the heaters evenly with about a 1 psi, or greater, average pressure in the areas of the PCR channels and the wax valves. Heater wire bonds can be protected from damage so as not to interfere with system motion. Registration from heater to cartridge and from cartridge to optical path centers can be within ± 0.010 inches. The reader can mechanically cycle a minimum of about 80,000 motions without failure.

Referring now to the one or more lysis heaters included in the system, the heaters for each of the 24 lysis stations can be individually software controlled. The lysis ramp times (e.g., the time that it takes for the water in a lysis tube to rise from a temperature of approximately 2.5° C. to a given temperature) can be less than 120 seconds for a rise to 50° C. and less than 300 seconds for a rise to 75° C. The lysis temperature (e.g., as measured in the water contained in a lysis tube) can be maintained, by the lysis heaters, within $\pm 3^\circ$ C. of the desired temperature. The accessible lysis temperature range can be from about 40° C. to about 82° C. Each of the lysis heaters may draw about 16 Watts or more of power when in operation. The lysis heater can be designed to maximize the thermal transfer to the lysis tube and also accommodate the tolerances of the parts. The lysis heaters can permit the lysis tubes to be in direct contact with the magnets (described in more detail herein). The lysis heaters may be adjustable in the horizontal plane during assembly and may not interface with the installed covers of the system.

Referring now to magnets included in the system, the lysis and magnet related mechanisms can fit beneath the rack and may not interface with rack insertion or registration. The magnets may be high-flux magnets (e.g., have about a 1,000 gauss, or greater, flux as measured within a given lysis tube) and be able to move a distance sufficient to achieve magnetic bead separation in one or more of the lysis tubes filled to a volume of 900 μ L. The magnets can be software-controllable at movement rates from about 1 mm/sec to about 25 mm/sec. The wiring, included as part of the heater and controller assemblies, can be contained and protected from

potential spills (e.g., spills of the lysis tubes). The magnets can be located about 1.25 inches or greater from the bottom of the lysis tube when not in use and can be retained in such a manner as to maximize contact with the lysis tube while also preventing jamming.

In some embodiments, the system enclosure includes a semi-transparent lid (e.g., with opaque fixtures and/or hardware) in the front of the instrument to allow users to view instrument functions. The lid can include a company and/or product logo and a graspable handle (e.g., enabling the user to raise the lid). When closed, the lid can have an opening force no greater than 15 pounds (e.g., when measured tangential to door rotation at the center of the bottom edge of the handle) and can lock in the open (e.g., "up") position such that no more than about 5 lbs. of force (e.g., applied at the handle and tangential to door rotation) is required to overcome the handle lock and return the lid to the closed position. The lid can include two safety lid locks that are normally locked when power is not applied and can allow the system to monitor the state (e.g., open or closed) of the lid. The lid can be designed such the lid does not fall when between the open and closed positions. the enclosure can include a power switch located on the right side of the instrument. A power cord can protrude from the enclosure in such a way that positioning the instrument does not damage the cords or cause accidental disconnection. The enclosure can prevent the user from coming in contact with, for example, moving part, high magnetic fields, live electrical connections, and the like. The enclosure can include four supporting feet, located on the underside of the enclosure, to provide a clearance of about 0.75 inches or more between the underside of the enclosure and the table top. The enclosure can include a recessed area with access to external accessory connections such as the display port, the Ethernet port, the 4 USB ports, and the like.

Referring now to the cooling sub-system included in the PCR system, an air intake can be provided in the front of the unit and an air exhaust can be provided in the rear portion of the top of the unit. Intake air can pass through the air intake and through a filter element (e.g., a removable and washable filter element). The cooling sub-system can maintain an interior air temperature (e.g., the temperature as is measured at the surface of the reagent strips, such as the reagent strips numbered 1, 12, and 24, at the surface of the PCR cartridges, and the like) about 10° C. higher, or less, than the ambient air temperature. The cooling subsystem can maintain the internal air temperature at or below about 32° C. One or more cooling fans included as part of the cooling subsystem may require about 5.7 Watts, or less, of power per fan.

In some embodiments, the system can include covers on internal subassemblies (with the exception of the gantry). The covers can be cleanable with a 10% bleach solution applied with a soft cloth without significant degradation. The covers can supply a safety barrier between a user and the electronic and moving mechanical assemblies included in the system. The covers on the internal subassemblies can be designed to maximize cooling of the internal subassemblies by maximizing airflow under the covers and minimizing airflow above the covers. The covers can be removable by a service technician and can match the color and texture of the enclosures.

In some embodiments, the system can be designed to operate within a temperature range of about 15° C. to about 30° C. and in a non-condensing relative humidity range (e.g., about 15% to about 80% relative humidity). The analyzer can be designed to perform without damage after

exposure to storage at no less than -20° C. for 24 hours or less, storage at no greater than 60° C. for 24 hours or less, and/or storage at about 50,000 feet or less (e.g., 3.4 inches of Hg) for 24 hours or less. The system can be designed with provisions to prevent motions that could damage the instrument during shipping. It can conform to the shipping standards set forth in ASTM D 4169-05, DC 12 and can be designed to allow the baseplate to be securely mounted to a shipping pallet. The racks and the enclosure of the instrument are designed not to degrade or be damaged by daily cleaning with a 10% bleach solution. The power to subassemblies of the system can be supplied by internal power supplies. Exemplary power supplies can receive, as input, about 1590 watts at about 90 to about 264 Vac at between about 47 and about 63 Hz and supply about 1250 watts of output to the subassemblies.

In some embodiments, the system can include a power switch (e.g., a rocker-type switch), located on the right side of the instrument, one or more interface components, and/or one or more interface ports. For example, the system can include an LCD display monitor that is 15 inches, has 1280x1024 pixel resolution and 16-bit color. The system can also include other display monitors such as ones with increased size, resolution, and/or color depth. The LCD display can be connected to the system via a VGA connection. The system can include a white, 2 button USB mouse, a white USB keyboard, a black SIT power cable, and an un-interruptible power supply, with feedback through USB. The system can also include a USB color printer, 2 USB cables (e.g., one for the printer and one for the UPS). The system can include exemplary interface ports, such as, 4 USB ports (e.g., to connect to a pointing device, printer, keyboard, UPS, LIS), 1 VGA port (e.g., for connection to the LCD display), and 1 Ethernet port (e.g., for PC connectivity) located on the left side of the enclosure. An IEC/EN 60320-11C14 power port can be included on the right side of the enclosure.

In some embodiments, the system can include features directed at increasing the safety of a user. For example, door interlocks can be included to prevent user access while the gantry is in motion and/or while other non-interruptible processes are underway. The system can be designed to minimize or eliminate the presence of user-accessible dangerous corners and/or edges on the instrument and designed such that metal parts are properly electrically grounded. Sheet metal or plastic covers can be included over mechanical and electrical components as necessary to protect a user from moving parts and/or live electrical parts and to protect the electronics and motors included in the system from, for example, spills.

Example 19: Exemplary Optics

Described herein are exemplary specifications related to the design of optics used in a PCR Analyzer and/or System. Additional information related to the PCR System is described elsewhere herein. The optical detection system included in the PCR System can be a 12-lane two-color detection system for monitoring real-time PCR fluorescence from a 12-lane microfluidic PCR cartridge. The system can include excitation lights (e.g., blue and amber LED light sources), one or more band pass filters, and one or more focusing lenses. The emitted fluorescence light from the PCR reactor (e.g., included in the microfluidic cartridge) is captured through a pathway into a focusing lens, through a filter, and onto a photodiode. Included in the system, for

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each PCR lane, are dedicated, fixed individual optical elements for each of the two colors interrogated.

In some embodiments, the limit of detection is 20 DNA copies per reaction of input PCR reaction mix with a minimum signal to base value of 1.15. The 2 color fluorescence system can be used with, for example, FAM (or equivalent) and Cal Red (or equivalent). The system can have the ability to collect fluorescence data in about 100 ms to about 600 ms at the maximum rate of one data point every about two seconds. When collecting data from a PCR lane, LEDs in adjacent lanes increase the signal in the lane being sampled by less than about 1% (e.g., 0.5%). The noise of the detection can be less than about 1% of the maximum signal. The lane-to-lane fluorescence variability with a fluorescence standard (e.g., part #14000009) can be within Cv of 30% for both FAM and Cal Red, when measured using the dark-current-corrected-fluorescence-slope. The average dark current-corrected-fluorescence-slope for the optical block with 12 lanes can be between about 30 mV to about 90 mV/(% blue LED power) for FAM using the fluorescence standard (Part #14000009). The average dark current-corrected-fluorescence-slope for the optical block with 12 lanes should be between about 75 mV to about 300 mV/(% amber LED power) for Cal Red using the standard fluorescence cartridge (Part #14000009). The storage excitation power for each channel can be independently varied by software from about 5% to about 100%. There may be no source of light activated inside the reader to affect the fluorescence reading. In some embodiments, turning room lights on or off does not affect the optical readings.

In some embodiments, the system can include an optical block with 12 repeats of 2-color fluorescence detection units at a pitch of about 8 mm. The optical detection block can be positioned on top of the microfluidic cartridge, with excitation and emission travelling through the PCR windows of the microfluidic cartridge. The apertures of the optical block can align with the PCR reactor within about ± 200 microns. An optical electronics board containing the LEDs and Photodetectors can be mated flush with the top of the optics block with each of the photodetectors recessed into the bores of its corresponding optical lane. When the microfluidic cartridge is installed in the system, the optical block can be used to deliver a force of about 20 to about 30 lbs. over the active area of the microfluidic cartridge with an average pressure of at least about 1 psi.

The optical block can be made of aluminum and surfaces present in the optical path lengths can be anodized black, for example, to minimize auto-fluorescence as well as light scattering. An aperture plate having 12 slits, each slit about 10 mm in length and 1 mm wide, can be used, for example, to limit the size of the excitation light spots as well as reduce background fluorescence. The thickness of the optics block can be about 1.135 ± 0.005 inches. The bottom surface of the optics block can be planar within ± 1 mil to provide uniform pressure over the micro fluidic cartridge. The apertures should be kept clean and free of debris during manufacturing of the optics block and assembly of the optics block into the system.

In some embodiments, the system can include excitation optics with an angle of excitation path equal to 55 ± 0.5 inches with respect to normal of the PCR cartridge surface. One exemplary arrangement of optical elements in the excitation path, in order, is LED, lens, filter, aperture, and PCR sample. The system can use a Plano-convex excitation lens (e.g., PCX, 6x9, MgF2TS) oriented with the flat side toward the PCR sample. Included in the optics are one or more excitation paths with tapers that can be designed such

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that the lens and filter can be placed inside the bore to provide a light spot bigger than the aperture plate. The location of the LED and the sample can be fixed as the design can include a fixed available optical block thickness. The location of the lens and the filter can be determined to provide a excitation spot size of about 6 mm along the length of a PCR lane. The excitation optics can include an LED such as Luxeon Part # LXX2-PB 14-NO0 (e.g., for FAM excitation) that includes a center wavelength of about 470 nm (blue) with a half band width of about 75 nanometers, or less (e.g., for FAM excitation). The excitation optics can also include an LED such as Luxeon Part # LXX2-PL12-Q00 (e.g., for Cal red excitation) that includes a center wavelength of 575 nm (amber) with a half band width of about 75 nanometers, or less (e.g., for Cal Red excitation). The LEDs used in the excitation optics can remain stable for about 5 years or more or about 10,000 cycles.

The system can include emission optics with an angle of emission path equal to about 15 ± 0.5 inches with respect to normal of the PCR cartridge surface. One exemplary arrangement of optical elements in the emission path, in order, is PCR sample, aperture, filter, lens, and photodetector. The emission lens can be plano-convex (e.g., PCX, 6x6 MgF2TS) with the flat side toward the photodetectors. The emission optics can include one or more bores, for the emission path, with tapers that can be designed so as to maximize detected light while enabling snug placement of the filters and lenses. The location of the photodetectors with respect to the sample can be fixed as the design can include a fixed available optical block thickness. The location of the lens and the filter can be determined so as to provide an emission spot size of 6 mm along the length of a PCR lane. An exemplary photodetector that can be used in the emission optics is the Hamamatsu Silicon Photodetector with Lens, S2386-18L.

In some embodiments, the system can include one or more filters with diameters of about 6.0 ± 0.1 mm, thicknesses of about 6.0 ± 0.1 mm, clear apertures with diameters of less than or equal to about 4 mm. The filters can include a blackened edge treatment performed prior to placement in a mounting ring. If present, the mounting ring can be metal and anodized black. The filters can be manufactured from optical glass with a surface quality that complies with F/F per Mil-C-48497A, and AOI of about 0 deg, a $\frac{1}{2}$ cone AOI of about +8 deg, and can be humidity and temperatures stable within the recommend operating range of the system. An exemplary filter can be obtained from Omega Optical Brattleboro, Vt. 05301.

The system can include one or ore FITC Exciter Filters (e.g., PN 14000001) with an Omega part number 481AF30-RED-EXC (e.g., drawing #2006662) used, for example, in FAM excitation. These filters can have a cut-on wavelength of about 466 ± 4 nm and a cut-off wavelength of about 496 ± 0.4 nm. The transmission of filters of this type can be greater than or equal to about 65% of peak. These filters can have a blocking efficiency of greater than or equal to OD4 for wavelengths of ultraviolet to about 439 nm, of greater than or equal to OD4 for wavelengths of about 651 nm to about 1000 nm, of greater than or equal to OD5 for wavelengths of about 501 nm to about 650 nm, and of greater than or equal to OD8, in theory, for wavelengths of about 503 nm to about 580 nm.

The system can include one or more Amber Exciter Filters (e.g., PN 14000002) with a part number 582AF25-RED-EXC (e.g., drawing #2006664) used, for example, in Ca Red excitation. These filters can have a cut-on wavelength of about 569 ± 5 nm and a cut-off wavelength of about 594+

0/-5 nm. The transmission of filters of this type can be greater than or equal to about 70% of peak. These filters can have a blocking efficiency of greater than or equal to OD8, in theory, for wavelengths of about 600 nm to about 700 nm.

The system can include one or more FITC Emitter Filters (e.g., PN 14000005) with a part number 534AF40-RED-EM (e.g., drawing #2006663) used, for example, in FAM emission. These filters can have a cut-on wavelength of 514+/-2 nm and a cut-off wavelength of 554+/-5 nm. The transmission of filters of this type can be greater than or equal to about 70A% of peak. These filters can have a blocking efficiency of greater than or equal to OD5 for wavelengths from ultraviolet to about 507 nm, of greater than or equal to OD8, in theory, from about 400 nm to about 504 nm, and of greater than or equal to OD4 avg. from about 593 nm to about 765 nm.

The system can include one or more Amber Emitter Filters (e.g., PN 14000006) with a part number 627AF30-RED-EM (e.g., drawing #2006665) used, for example, in Cal Red emission. These filters can have a cut-on wavelength of 612+5/-0 nm and a cut-off wavelength of 642+/-5 nm. The transmission of filters of this type can be greater than or equal to about 70% of peak. These filters can have a blocking efficiency of greater than or equal to OD5 for wavelengths from ultraviolet to about 605 nm, of greater than or equal to OD8, in theory, from about 550 nm to about 600 nm, and of greater than or equal to OD5 avg. from about 667 nm to about 900 nm.

Example 20: Exemplary 3-Layer Cartridge

Described herein are exemplary specifications used to design and assemble the microfluidic cartridge as well as exemplary instructions on the use of the cartridge in, for example, the system described herein. In some embodiments, the cartridge can have a maximum limit of detection equal to 20 copies per reaction volume (e.g., 20 copies/4 μ l), with a target detection of 10 copies per reaction volume. The cartridge can perform 45 reactive cycles in 40 minutes or less (e.g., 45 cycles in 40 minutes, 45 cycles in 20 minutes, 45 cycles in 15 minutes, or the like). The cartridge can utilize two color detection using, for example, the FAM (or equivalent) and CAL RED (or equivalent) fluorescent dyes. Results obtained using the cartridge have been compared with the results obtained using standard real-time PCR instruments.

In some embodiments, the Cartridge can be a one-time use, disposable cartridge that can be disposed of according to typical laboratory procedures. The cartridge can be 4.375 inches long and 2.800 inches wide, with a thickness of 0.094+/-0.005 inches. The cartridge can include features that allow the cartridge to interface with, for example, the system described herein. Exemplary interfacing features include PCR channel walls and the top of the micro-substrate over the PCR channel that are well polished (SPI A1/A2/A3), enabling easy transfer of excitation and emission light between the PCR reactor (e.g., contained in the cartridge) and the detection system (e.g., the analyzer). The cartridge can include a thermal interface, located on the bottom of the cartridge, for interfacing with the analyzer. The thermal interface can have a thin laminate (e.g., less than 150 microns thick, 100 microns thick, or the like) to encourage heat transfer from the heater wafer to, for example, the PCR channels of the cartridge.

The cartridge can include one or more mechanical interface with, for example, the analyzer. For example, the cartridge can have a notch in one or more of the corners that can mate with a corresponding shape on the heater module

of the analyzer. The notch and corresponding shape can enable the cartridge to be placed only one way in the tray of, for example, the system described herein. In some embodiments, the cartridge has a single notch in one of the corners, with the remaining three corners have a minimum radius of 1 mm to facilitate placement of the cartridge in the analyzer. During use (e.g., when placed in a system described herein and performing a function such as PCR), the cartridge can be pressed, on one side, by the optics block, against the heater wafer (positioned against the opposite side), with a pressure of about 1 psi or greater (e.g., 0.99 psi, 1.2 psi, or the like). When located in the tray of the analyzer, the cartridge can have an alignment slop of +/-200 microns to enable a user to easily place and remove the cartridge from the analyzer tray. The cartridge can have two ledges, that are each 1 mm wide and located along the two long edges of the cartridge, to enable the heating surface to extend below the datum of the tray.

In some embodiments, the cartridge can have the following functional specifications. The cartridge can include an inlet hole that is, for example, cone-shaped with a height of 1 mm from the top surface of the cartridge. The cone can have an inner diameter of 3 mm at the top of the cone and can taper down to a diameter that matches the width of a microchannel (e.g., an inlet channel) that the inlet cone is fluidly connected to. the inlet channel can fluidly connect the inlet hole to a PCR reactor that has an interior volume of, for example, about 4.25 μ l to 4.75 μ l (e.g., 4.22 μ l, 4.5 μ l, 4.75 μ l, or the like). An outlet microfluidic channel can fluidly connect the PCR reactor to an overflow chamber. The cartridge can also include an outlet vent hole.

The input PCR sample (e.g., a reaction mixture) can be between about 6.0 and 7.0 μ l per PCR lane (e.g., 5.9 μ l per lane, 6.4 μ l per lane, 7.1 μ l per lane, or the like) and can be introduced into the cartridge through the inlet hole by, for example, a pipette. The reaction mixture can be transported, via the inlet channel, to the PCR reactor where the reaction mixture can be isolated (e.g., sealed off by valves) to prevent evaporation or movement of the reaction mixture during thermocycling. Once the mixture is sealed inside the chamber, the analyzer can initiate multiplexed real-time PCR on some or all of the reaction mixture (e.g., 4.5 μ l, an amount of fluid equal to the inner volume of the reaction chamber, or the like).

The microfluidic substrate of the cartridge can include one or more of the following specifications. The material of the microsubstrate can be optically clear (e.g., have about 90% or greater optical transmission, be 3 mm thick, comply with ASTM D1003, and the like), have auto-fluorescence that is less than that emitted by 2 mm thick ZEONOR 1420R, and have a refractive index of about 1.53 (ASTM D542). The material of the microsubstrate can be amenable to the injection molding of features required for the microfluidic network of the cartridge. The material is preferably compatible with all PCR gents and can withstand temperatures of up to about 130° C. for about 5 minutes or more without yielding or melting. The cartridge can include fiducials, recognizable by HandyLab manufacturing equipment, located in one or more (preferably two) of the corners of the substrate. The cartridge can include fluidic components (e.g. microchannels, valves, end vents, reagent inlet holes, reaction chambers, and the like) necessary to perform the functions of the cartridge (e.g., PCR).

Additional features of the substrate material can include one or more of the following: Minimum clearances of about 1 mm can be designed between functional features to ensure sealing success (e.g., to the analyzer), and to allow simpli-

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fied fixturing during assembly. The cartridge can include dogbones under small fluid path ends to, for example, increase mold life. The bottom of the micro tool surface can be roughened (e.g., by vapor hone, EDM, or the like). The substrate material can be capable of adhesion by a label.

In some embodiments, the sealing tape used in the cartridge can include one or more of the following specifications: Laminate can be easily applied to the bottom of the microfluidic substrate. Material of the laminate is preferably pin-hole free. The material and adhesive is preferably compatible with the PCR reaction chemistries. The laminate material and glue used should not auto-fluoresce. The material can withstand up to 130° C. for 5 minutes without losing adhesion, yielding, melting, or causing undue stresses on the cartridge. Bubbles should not form in the adhesive layer upon heating (e.g., to 130° C. for 5 minutes) after application to the microsubstrate. The laminate should be less than 5 mills thick to, for example, enable rapid heat transfer.

The high temperature wax included in the cartridge can have the following characteristics. The wax should have a melt point of about 90+3° C. (e.g., 87° C., 90° C., 93.1° C., or the like), be biocompatible with PCR reactions, have wettability with microsubstrate material, and have a melt viscosity range, for example, of about Viscosity at 100° C.=20 mm²/s and Hardness at 25° C.=8 dmm. The main label of the cartridge can have the following characteristics. It can have a thickness of 2-4 mils, have suitable bondability to micro features and seal around the valves, include cuts for one or more PCR windows, and a ta (free from adhesive) for aiding in removal of the cartridge from the analyzer. The main label can also have abrasion resistance on the top surface, and be printable. The main label can have an upper and lower alignment pattern for the label to completely cover the valve holes for proper operation of the valves.

The cartridge can include a barcode label applied to the top of the cartridge that is readable by a barcode reader (e.g., the barcode reader included in the analyzer) while the cartridge is installed in the analyzer. The barcode label can include the produce name, lot #, expiration date, bar code (2D) and may be printed on. In addition, or in the alternative, a barcode may be applied directly to the main cartridge label using a laser or inkjet type printer.

The packaging that the cartridge is included in can include one or more of the following: package label, carton, carton label, and/or operating instructions. The packaging can be printed on or label attachable, placed inside of a plastic bag, shrink/stretch wrap bag, or the like, and can be stacked in groups of 24. The cartridge bagging without a critical seal should be kept free from dust contamination.

The cartridge can include one or more valves (e.g., temperature controlled, wax-containing valves) for starting, stopping, and/or controlling the flow of material inside the cartridge. The wax contained in the valves can be free of trapped air bubbles that have a diameter greater than half the width of the valve channel. The valve channel can have an air pocket. The wax may not intrude into the fluid path prior to activation. The wax can be filled to the start of the flare to the fluid path.

The cartridge can include micro channels and holes such that the holes are of a size and shape to enable easy, leak-free interfacing with a 175 µl pipette tip. In some examples, the holes size is between about 200 µm and about 4000 µm in diameter. The microchannels can be between about 50 µm and about 1500 µm wide and between about 50 µm and 1000 µm high.

The cartridge can include valves for controlling the flow of fluid within the cartridge (e.g., through the microchan-

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nels, reactor chambers, and the like). The valve edges, steps, and general geometry can be designed to encourage exact flow and/or stoppage required during wax load. The valve geometry can be designed to accommodate limitations of wax dispensing equipment (e.g., ±25% of 75 nL volume). In some embodiments, step down air chambers on the valves are funnel shaped to aid wax loading and the remaining geometry diminishes from the bottom of the funnel to the end point where the wax stops. The path where the valves are to flow into and block, during use, can be narrow enough (e.g., 150-200 microns wide and deep) and have enough length to effectively seal when the valves are activated during use. The valve wax temperature can be about 90° C. When in use to block a portion of a microchannel, the valves can seal to prevent evaporation of fluid and/or physical migration of fluid from the PCR reactor during thermocycling.

The cartridge can include one or more PCR regions for performing PCR on a sample. The channel in the PCR region (e.g., PCR reactor) can be designed such that the temperature of the contents of the channel remain uniformly within about 1° C. of the anneal temperature. The channel walls can have a polish of SPI A1/A2/A3.

In some embodiments, the cartridge is designed to be able to perform diagnostic tests within a temperature range of about 59° F. to about 86° F. (about 15° C. to about 30° C.) and a humidity range of about 15% relative humidity to about 80% relative humidity. The cartridge is designed to be safe and functional when used indoors, used at an altitude of 2000 m or less, and used under non-condensing humidity conditions (e.g., maximum relative humidity of 80% for temperatures up to 31° C. decreasing linearly to 50% relative humidity at 40° C.).

In use, PCR product produced in the cartridge can remain in the used cartridge to, for example, minimize the likelihood of cross contamination. The cartridge can be designed such that a 4 foot drop of the cartridge, while in its packaging, will not damage the cartridge. The cartridge is designed to perform without damage after exposure to the following conditions. The cartridge should be stored at 4° C. to 40° C. for the rated shelf life. Exposure to temperatures between -20° C. and 4° C. or 40° C. and 60° C. should occur for no longer than 24 hours. The cartridge can withstand air pressure changes typical of air transport.

The cartridge can be labeled with the following information (e.g., to identify the cartridge, comply with regulations, and the like). The label can contain a "Research Use Only" label, if applicable, and a CE mark, if applicable. The label can contain the company name and logo (e.g., Handylab®), a part number (e.g., 55000009), a part name (12x Cartridge-nonvented), a lot number (e.g., LOT 123456), an expiration date (e.g., Jun. 6, 2015), space for writing, a barcode according to barcode specifications (described elsewhere), and/or "Handylab, Inc., Ann Arbor, Mich. 48108 USA".

The cartridge can be include in a carton that can contain information such as, a part number (e.g., 55000009), a part name (12x Cartridge-nonvented), a quantity (e.g., 24), a lot number (e.g., LOT 123456), an expiration date (e.g., June 2015), an optional UPC code, "Manufactured by Handylab, Inc., Ann Arbor, Mich. 48108 USA", a carton label to state storage limits, a CE mark (if applicable), and/or an AR name and address.

The cartridge packaging can include paper wrap to secure multiple cartridges together and clean package fill to prevent damage, for example, from vibration. The cartridge shipping carton can include features such as, compliance to ASTM 6159, carton may be stored in any direction, refrigeration or

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fragile labeling of the carton may not be required, and additional cold packs may not be required. The shelf life of the cartridge is 12 months or more.

The cartridge can comply with IEC 61010 (NRTL tested) and an FDA listing may be required for clinical distribution. Cartridges used in a clinical lab device may meet all quality system requirements. Cartridges used for research only in a commercial device may meet all HandyLab quality system requirements. Cartridges for research use only (Alpha or Beta testing) may be design/manufacturing traceable to a DHR (manufacturing record).

The foregoing description is intended to illustrate various aspects of the present inventions. It is not intended that the examples presented herein limit the scope of the present inventions. The technology now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. A system for processing a plurality of nucleic acid-containing samples, the system comprising:

a first module configured to extract nucleic acids from the plurality of nucleic acid-containing samples,

a second module configured to amplify the nucleic acid extracted from the plurality of nucleic acid-containing samples, the first and second modules comprising:

a bay configured to removably receive a housing comprising a plurality of process chambers that are maintained at a same height relative to one another when the housing is received in the bay, the plurality of process chambers aligned along a first axis when the housing is received in the bay, the bay comprising one or more complementary registration members configured to receive the housing in a single orientation when the housing is received in the bay, the first module further comprising a magnetic separator positioned to apply a magnetic force to a first side of the plurality of process chambers when the housing is received in the bay, the magnetic separator comprising one or more magnets aligned along a second axis parallel to the first axis when the housing is received in the bay, the one or more complementary registration members further configured to align the plurality of process chambers with the magnetic separator when the housing is received in the bay, and

the first module further comprising a heating assembly positioned adjacent to a second side of the plurality of process chambers opposite the first side when the housing is received in the bay, the heater assembly comprising one or more heaters aligned along a third axis parallel to the first axis when the housing is received in the bay, the heating assembly configured to heat a solution in the plurality of process chambers to between 50° C. and 85° C., the one or more complementary registration members configured to align the plurality of process chambers with the heater assembly when the housing is received in the bay; and

a liquid dispenser configured to move between a first location and a second location when the housing is received in the bay, the liquid dispenser configured to dispense at least a portion of the plurality of nucleic acid-containing samples and a plurality of magnetic binding particles when the housing is received in the bay and the liquid dispenser is in the first location, the liquid dispenser further configured to dispense

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the nucleic acid extracted from the plurality of nucleic-acid containing samples when the liquid dispenser is in the second location.

2. The system of claim 1, wherein a sample of the plurality of nucleic acid-containing samples corresponds with a process chamber of the plurality of process chambers when the housing is received in the bay.

3. The system of claim 1, wherein the liquid dispenser comprises one or more dispense heads configured to accept a pipette tip.

4. The system of claim 3, wherein the liquid dispenser comprises four dispense heads and the housing comprises twelve process chambers, each dispense head configured to dispense a plurality of magnetic binding particles and at least a portion of one sample of the plurality of nucleic acid-containing samples into one of the twelve process chambers when the housing is received in the bay.

5. The system of claim 1, further comprising a sample identification verifier configured to check an identity of each sample of the plurality of nucleic acid-containing samples, wherein the sample identification verifier is selected from the group consisting of an optical character reader, a bar code reader, and a radio frequency tag reader.

6. The system of claim 1, further comprising electronic circuitry configured to control operation of the magnetic separator, the heater assembly, and the liquid dispenser.

7. The system of claim 6, wherein the electronic circuitry is configured to cause the magnetic separator to apply a magnetic force to the plurality of process chambers when the housing is received in the bay.

8. The system of claim 6, wherein the electronic circuitry is configured to cause the heater assembly to apply heat to the plurality of process chambers when the housing is received in the bay.

9. The system of claim 6, wherein the electronic circuitry is configured to control motion of the liquid dispenser when the housing is received in the bay.

10. The system of claim 1, further comprising one or more processors and at least one input device coupled to the one or more processors, the at least one input device selected from the group consisting of: a keyboard, a touch-sensitive surface configured to accept input from a stylus or a user's finger, a microphone, a track-pad, a retinal scanner, a fingerprint reader, a holographically projected interface, and a mouse.

11. The system of claim 10, further comprising a communication interface coupled to the one or more processors, the communication interface being selected from the group consisting of: a serial connection, a parallel connection, a wireless network connection, a wired network connection, and one or more USB ports.

12. The system of claim 11, further comprising a data storage medium configured to receive data from the one or more processors, the at least one input device, and the communication interface, the storage medium being selected from the group consisting of: a hard disk drive, an optical disk drive, a flash-card, a USB-drive, and a CD-Rom.

13. The system of claim 12, further comprising at least one output device coupled to the one or more processors, the at least one output device being selected from a visual display, a printer, a holographic projection, and a speaker.

14. The system of claim 1, further comprising more than one bay, each bay configured to removably receive a housing comprising a plurality of process chambers.

15. The system of claim 1, wherein the number of nucleic acid-containing samples is twelve.

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16. The system of claim 1, further comprising an optical detection system configured to independently detect a plurality of fluorescent dyes at a plurality of different locations, wherein each fluorescent dye binds to a fluorescent polynucleotide probe or a fragment thereof.

17. The system of claim 16, wherein the optical detection system selectively emits light in an absorption band of the plurality of fluorescent dyes and selectively detects light in an emission band of the plurality of fluorescent dyes.

18. The system of claim 16, configured to carry out extraction, amplification, and detection of the plurality of nucleic acid-containing samples in less than an hour.

19. The system of claim 1, further comprising a heater substrate comprising at least one heat source configured to apply heat at one or more selected times in order to apply thermocycling operations sufficient to amplify the nucleic acid extracted from the plurality of nucleic acid-containing samples.

20. The system of claim 19, wherein the at least one heat source is configured to maintain a negligible temperature gradient across a reaction zone during the thermocycling operations, the reaction zone configured to receive the nucleic acid extracted from one of the plurality of nucleic acid-containing samples.

21. The system of claim 20, wherein the at least one heat source is configured to maintain a negligible temperature gradient across each of a plurality of reaction zones during the thermocycling operations, each reaction zone configured to receive the nucleic acid extracted from one of the plurality of nucleic acid-containing samples.

22. A system for processing a plurality of nucleic acid-containing samples, the system comprising:

a first module configured to extract nucleic acids from the plurality of nucleic acid-containing samples, the first module comprising:

a bay configured to removably receive a housing comprising a plurality of process chambers that are maintained at a same height relative to one another when the housing is received in the bay, the plurality of process chambers aligned along a first axis when the housing is received in the bay, the bay comprising one or more complementary registration members configured to receive the housing in a single orientation when the housing is received in the bay, a magnetic separator positioned to apply a magnetic force to a first side of the plurality of process chambers when the housing is received in the bay, the magnetic separator comprising one or more magnets aligned along a second axis parallel to the first axis when the housing is received in the bay, the one or more complementary registration members further configured to align the plurality of process chambers with the magnetic separator when the housing is received in the bay, and

a heating assembly positioned adjacent to a second side of the plurality of process chambers opposite the first side when the housing is received in the bay, the heater assembly comprising one or more heaters aligned along a third axis parallel to the first axis when the housing is received in the bay, the heating

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assembly configured to heat a solution in the plurality of process chambers to between 50° C. and 85° C., the one or more complementary registration members configured to align the plurality of process chambers with the heater assembly when the housing is received in the bay;

a second module configured to amplify the nucleic acid extracted from the plurality of nucleic acid-containing samples; and

a liquid dispenser configured to move between a first location and a second location when the housing is received in the bay, the liquid dispenser configured to dispense at least a portion of the plurality of nucleic acid-containing samples and a plurality of magnetic binding particles when the housing is received in the bay and the liquid dispenser is in the first location, the liquid dispenser further configured to dispense the nucleic acid extracted from the plurality of nucleic acid-containing samples into the second module when the liquid dispenser is in the second location.

23. The system of claim 22, wherein the liquid dispenser comprises one or more dispense heads configured to accept a pipette tip.

24. The system of claim 23, wherein the liquid dispenser comprises four dispense heads and the housing comprises twelve process chambers, each dispense head configured to dispense a plurality of magnetic binding particles and at least a portion of one sample of the plurality of nucleic acid-containing samples into one of the twelve process chambers when the housing is received in the bay.

25. The system of claim 22, further comprising electronic circuitry configured to control operation of the magnetic separator, the heater assembly, and the liquid dispenser.

26. The system of claim 22, further comprising one or more processors and at least one input device coupled to the one or more processors, the at least one input device selected from the group consisting of: a keyboard, a touch-sensitive surface configured to accept input from a stylus or a user's finger, a microphone, a track-pad, a retinal scanner, a fingerprint reader, a holographically projected interface, and a mouse.

27. The system of claim 22, wherein the first module comprises more than one bay, each bay configured to removably receive a housing comprising a plurality of process chambers.

28. The system of claim 22, wherein the number of nucleic acid-containing samples is twelve.

29. The system of claim 22, further comprising an optical detection system configured to independently detect a plurality of fluorescent dyes at a plurality of different locations, wherein each fluorescent dye binds to a fluorescent polynucleotide probe or a fragment thereof.

30. The system of claim 22, further comprising a heater substrate comprising at least one heat source configured to apply heat at one or more selected times in order to apply thermocycling operations sufficient to amplify the nucleic acid extracted from the plurality of nucleic acid-containing samples.

* * * * *

EXHIBIT 41



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(12) **United States Patent**
Williams et al.

(10) **Patent No.:** **US 10,625,262 B2**

(45) **Date of Patent:** ***Apr. 21, 2020**

(54) **INTEGRATED APPARATUS FOR PERFORMING NUCLEIC ACID EXTRACTION AND DIAGNOSTIC TESTING ON MULTIPLE BIOLOGICAL SAMPLES**

(58) **Field of Classification Search**

CPC B01L 3/502761; B01L 3/0275; B01L 3/5027; B01L 3/52; B01L 7/52;
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This patent is subject to a terminal disclaimer.

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(21) Appl. No.: **16/698,022**

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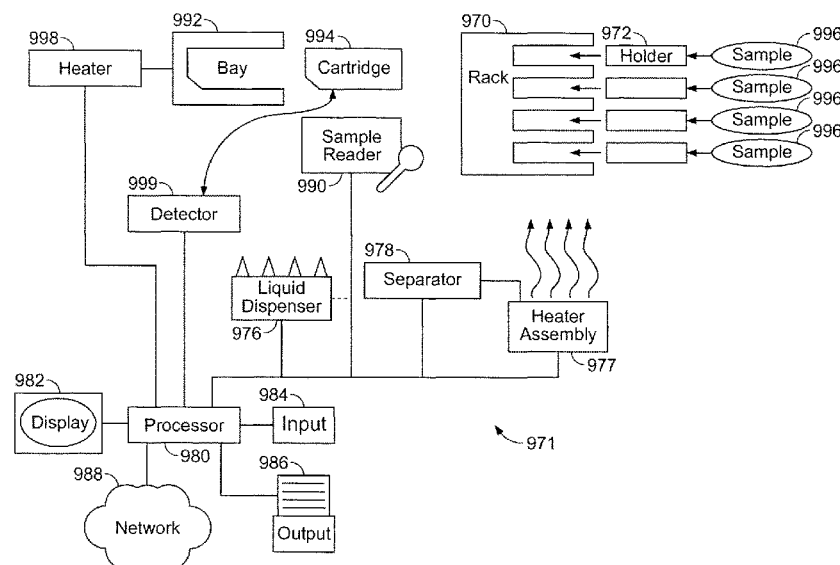
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(Continued)

(57) **ABSTRACT**

The technology described herein generally relates to systems for extracting polynucleotides from multiple samples, particularly from biological samples, and additionally to systems that subsequently amplify and detect the extracted polynucleotides. The technology more particularly relates to microfluidic systems that carry out PCR on multiple samples of nucleotides of interest within microfluidic channels, and detect those nucleotides.

24 Claims, 121 Drawing Sheets



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Related U.S. Application Data

- No. 14/941,087, filed on Nov. 13, 2015, now Pat. No. 10,071,376, which is a continuation of application No. 12/218,498, filed on Jul. 14, 2008, now Pat. No. 9,186,677, which is a continuation-in-part of application No. 11/985,577, filed on Nov. 14, 2007, now Pat. No. 7,998,708.
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- (52) **U.S. Cl.**
CPC **B01L 3/52** (2013.01); **B01L 7/52** (2013.01); **B01L 9/06** (2013.01); **B01L 9/527** (2013.01); **F16K 99/0001** (2013.01); **F16K 99/003** (2013.01); **F16K 99/0032** (2013.01); **F16K 99/0044** (2013.01); **F16K 99/0061** (2013.01); **B01L 2200/027** (2013.01); **B01L 2200/10** (2013.01); **B01L 2200/147** (2013.01); **B01L 2200/148** (2013.01); **B01L 2200/16** (2013.01); **B01L 2300/021** (2013.01); **B01L 2300/045** (2013.01); **B01L 2300/06** (2013.01); **B01L 2300/0627** (2013.01); **B01L 2300/0681** (2013.01); **B01L 2300/087** (2013.01); **B01L 2300/0816** (2013.01); **B01L 2300/0832** (2013.01); **B01L 2300/0867** (2013.01); **B01L 2300/0887** (2013.01); **B01L 2300/18** (2013.01); **B01L 2300/1822** (2013.01); **B01L 2300/1827** (2013.01); **B01L 2300/1861** (2013.01); **B01L 2400/0442** (2013.01); **B01L 2400/0481** (2013.01); **B01L 2400/0487** (2013.01); **B01L 2400/0611** (2013.01); **B01L 2400/0677** (2013.01); **B01L 2400/0683** (2013.01); **F16K 2099/0084** (2013.01); **G01N 35/026** (2013.01); **G01N 2035/00881** (2013.01); **G01N 2035/0425** (2013.01); **G01N 2035/0436** (2013.01)
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CPC B01L 9/06; B01L 9/527; F16K 99/0001; F16K 99/003; F16K 99/0032; F16K 99/0044; F16K 99/0061
See application file for complete search history.
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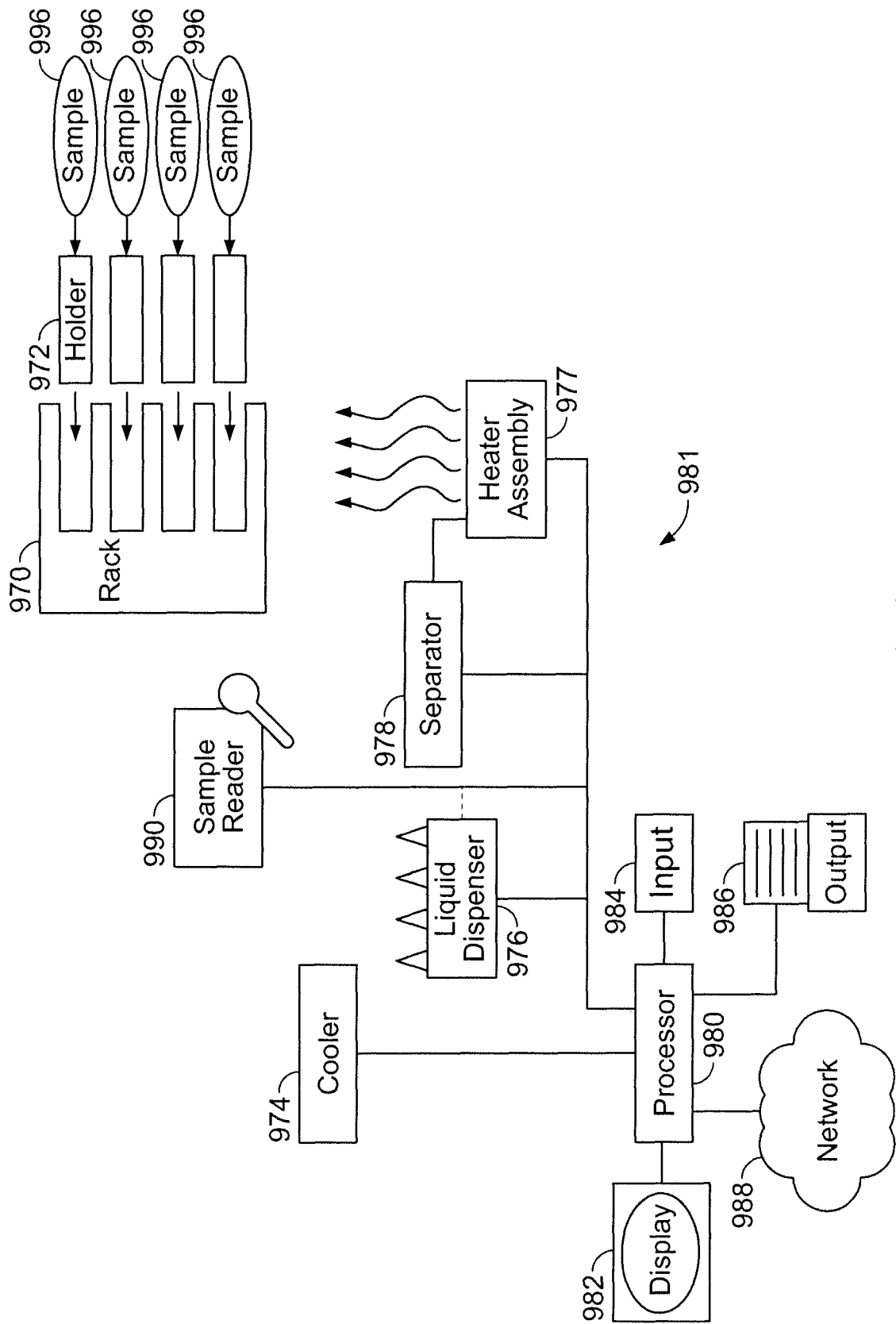


FIG. 1A

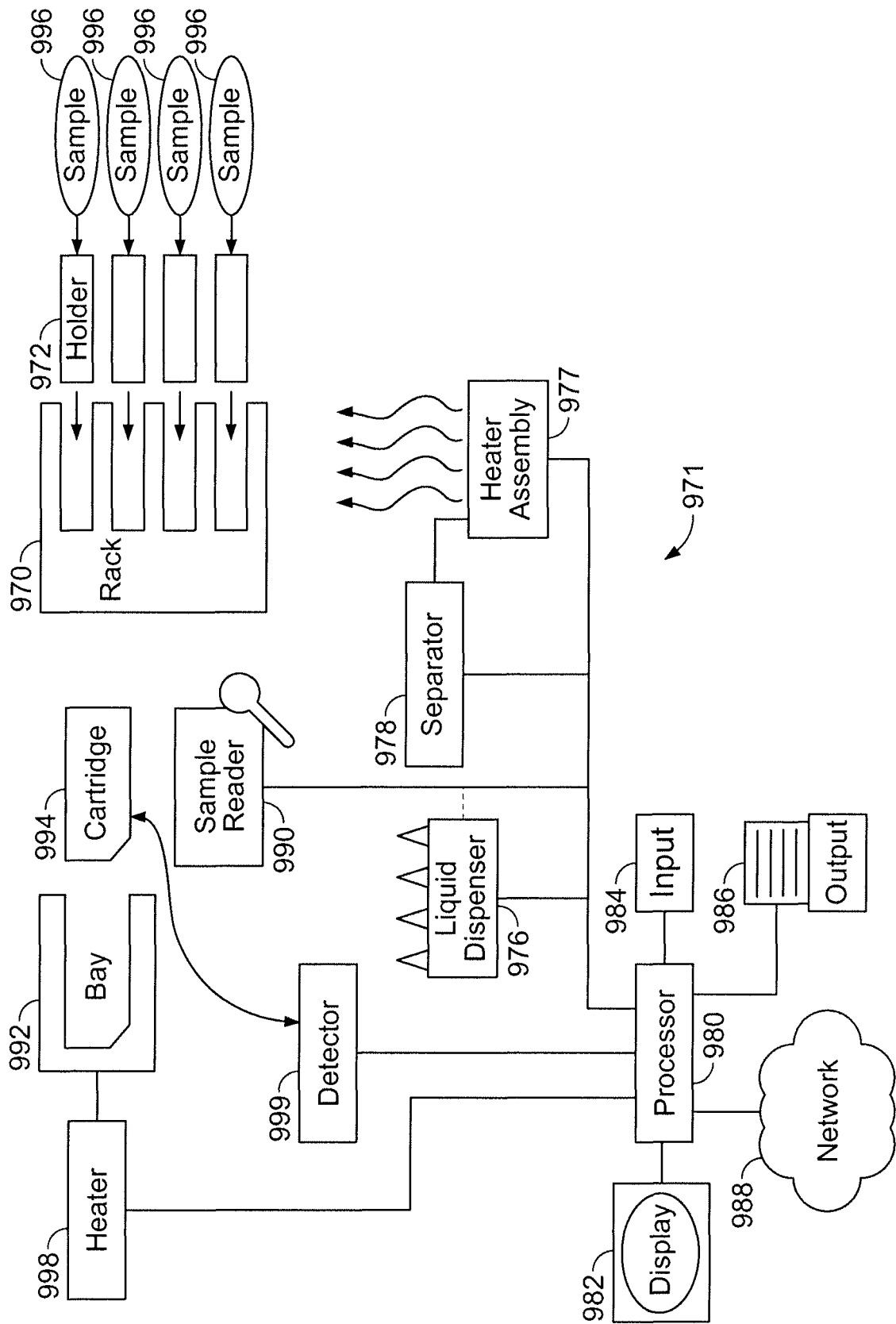


FIG. 1B

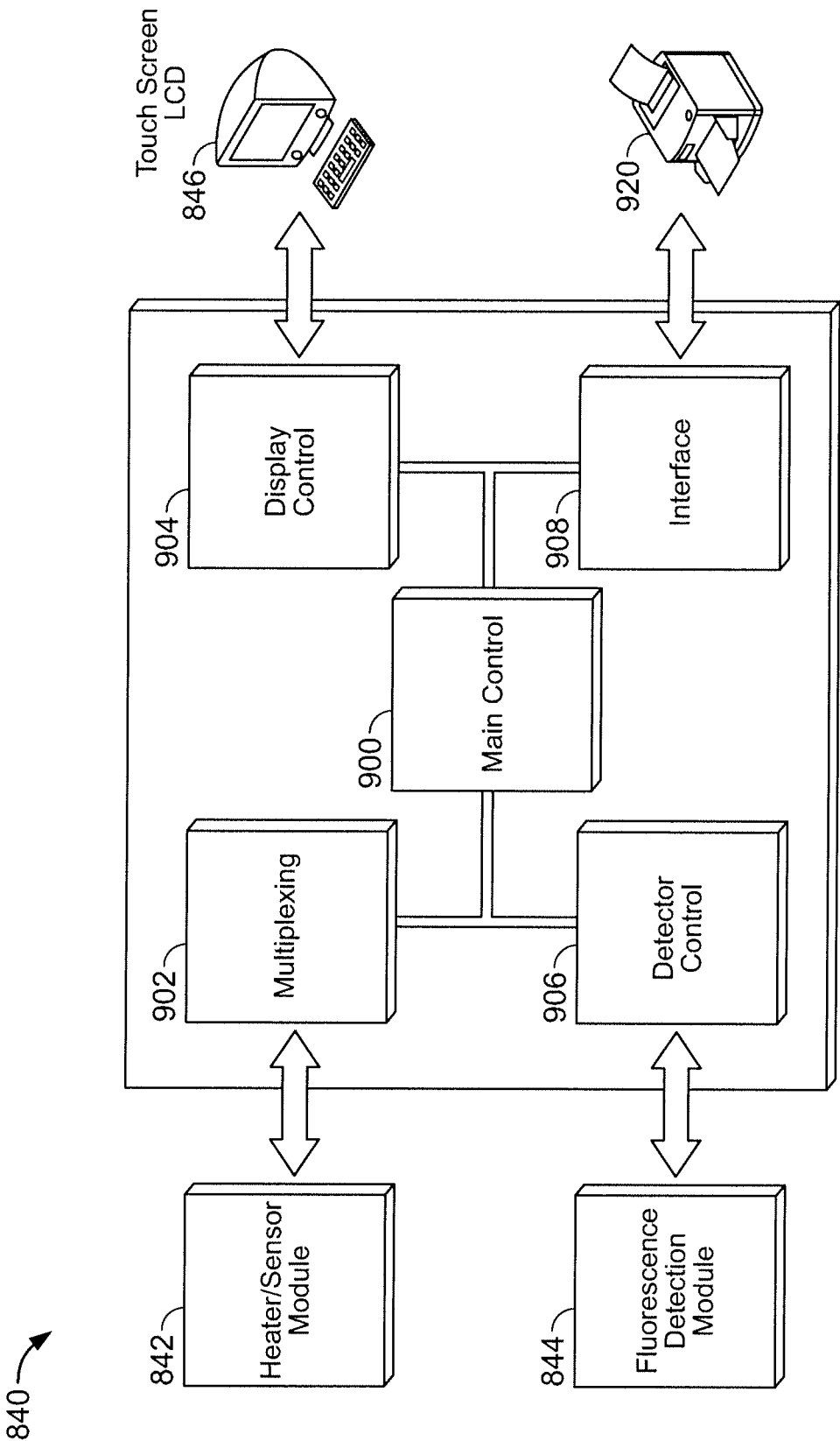


FIG. 2

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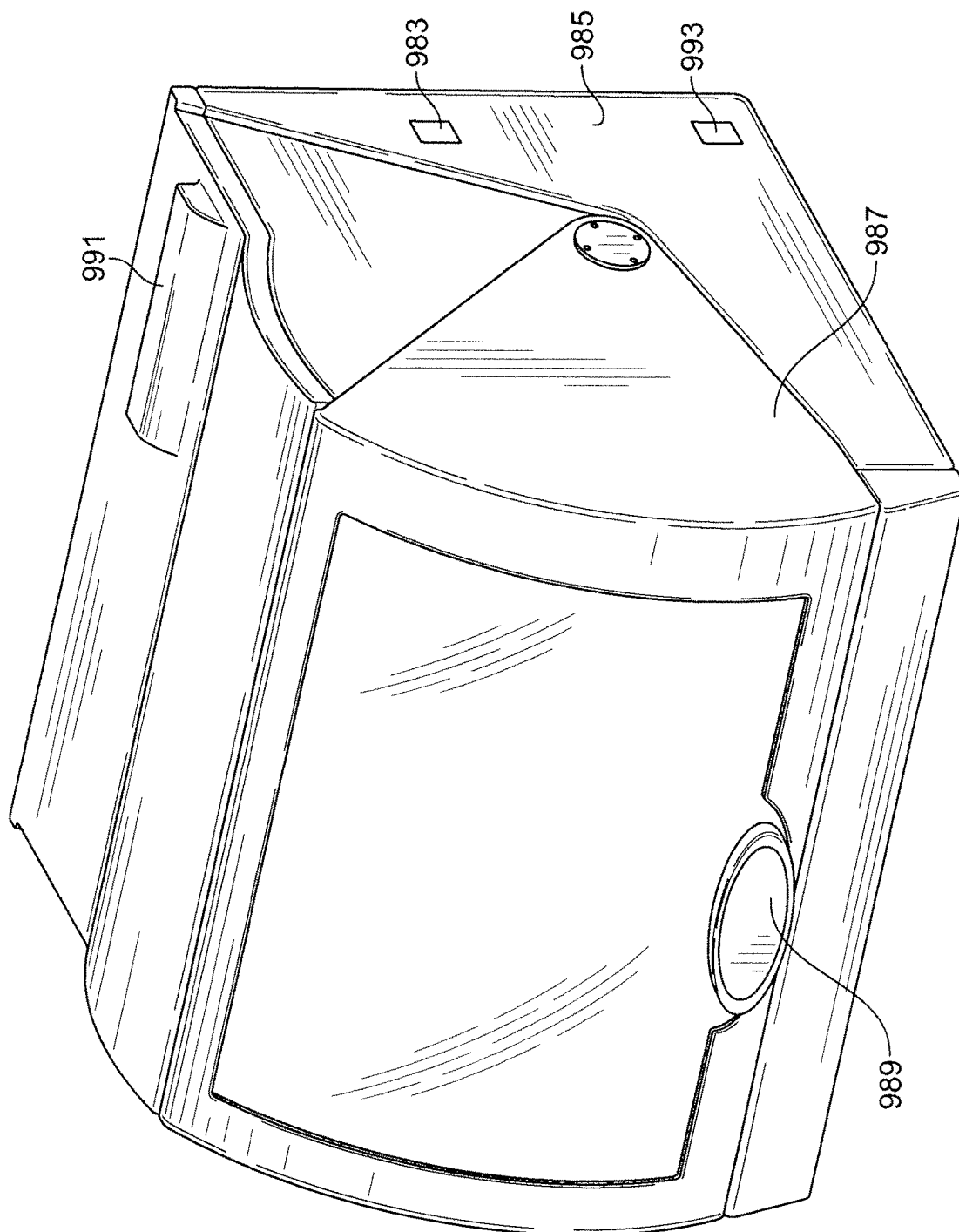


FIG. 3A

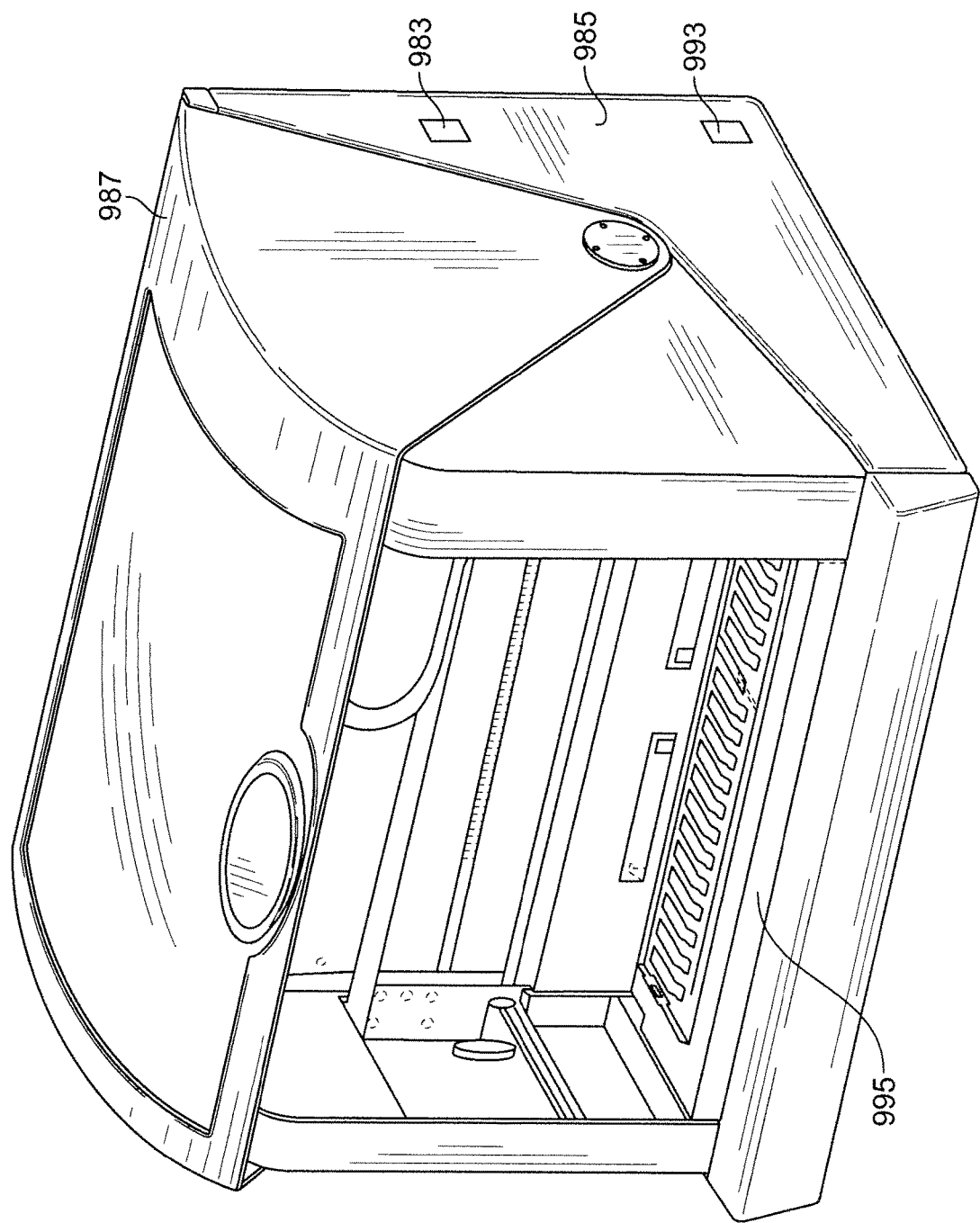
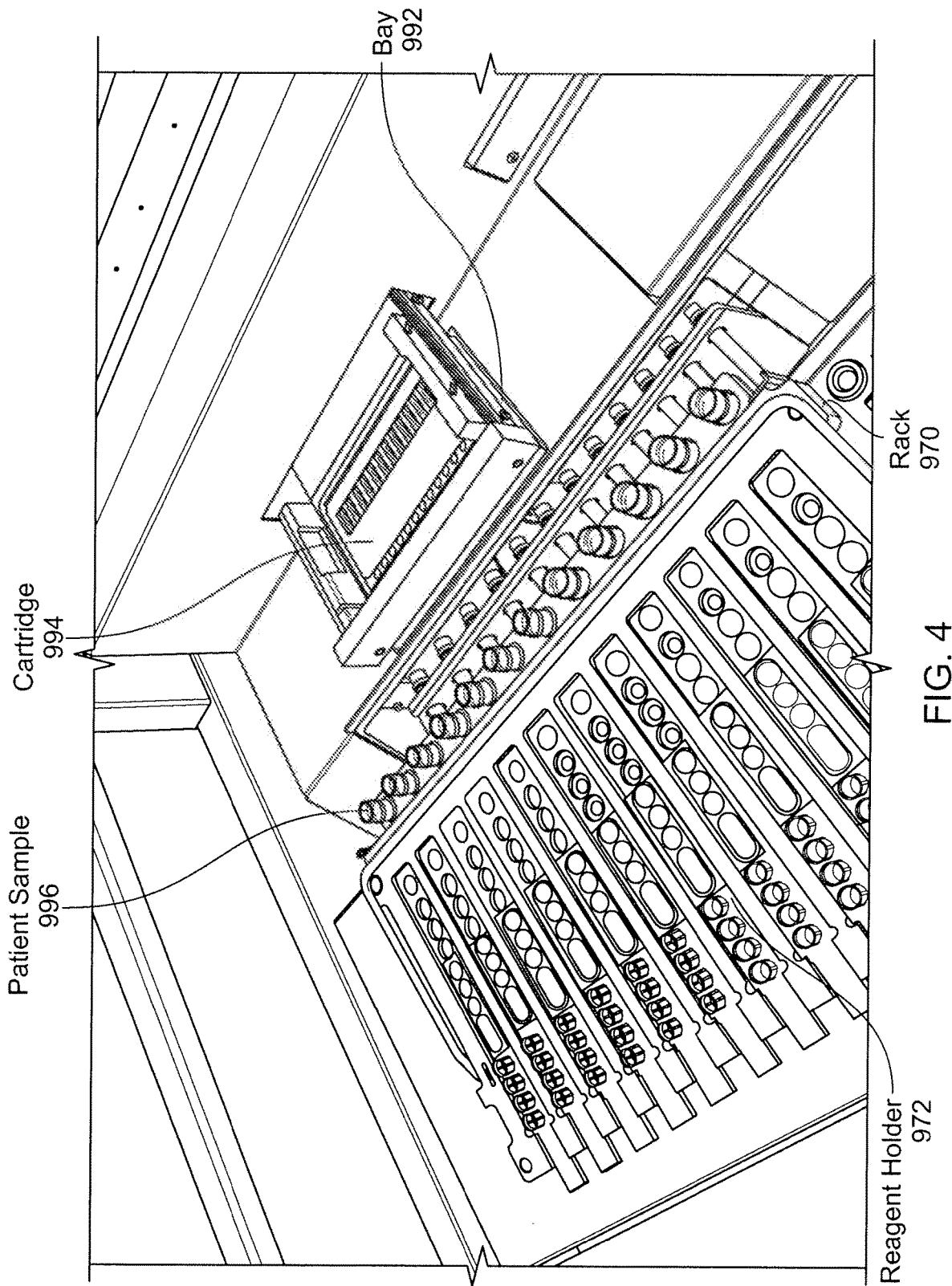


FIG. 3B



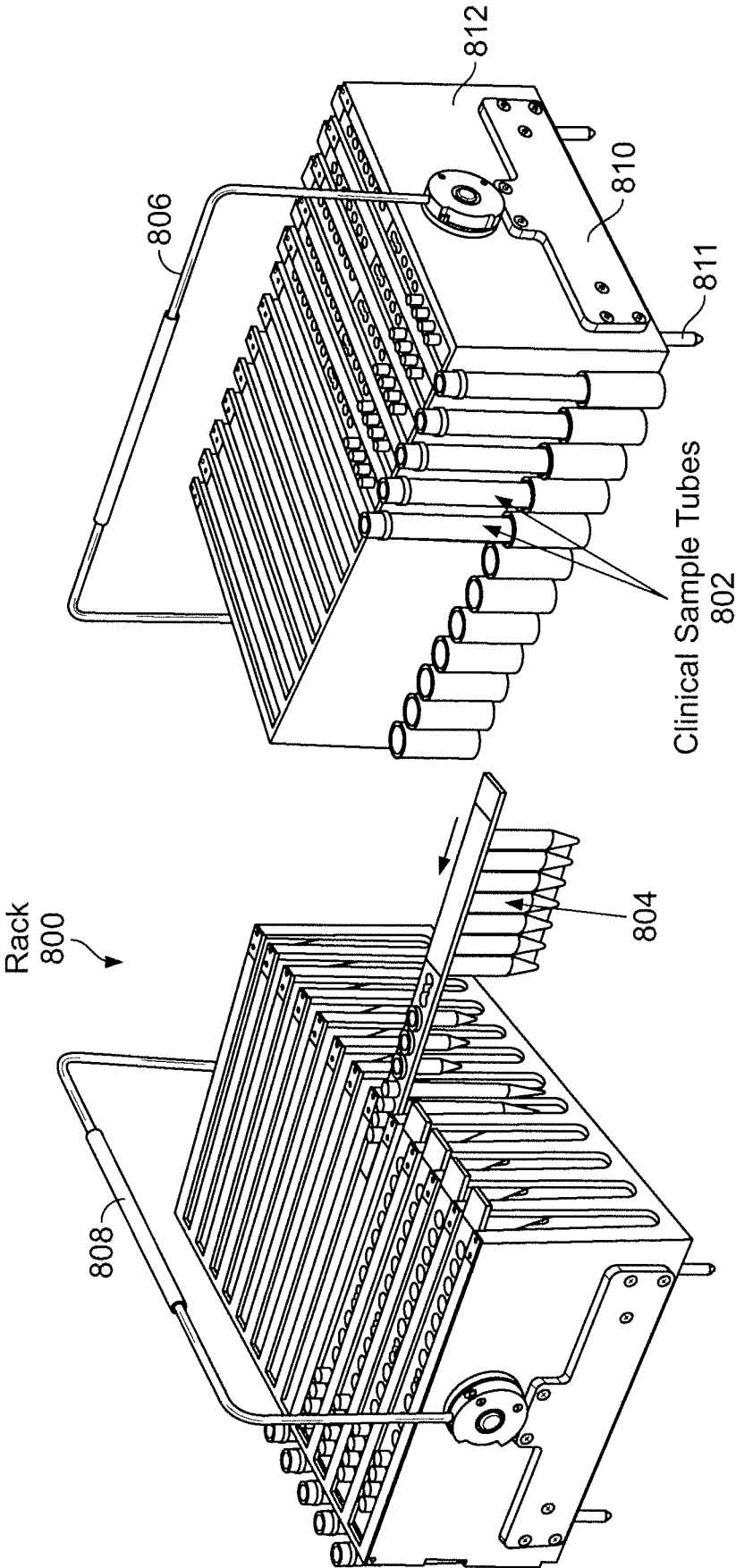


FIG. 5

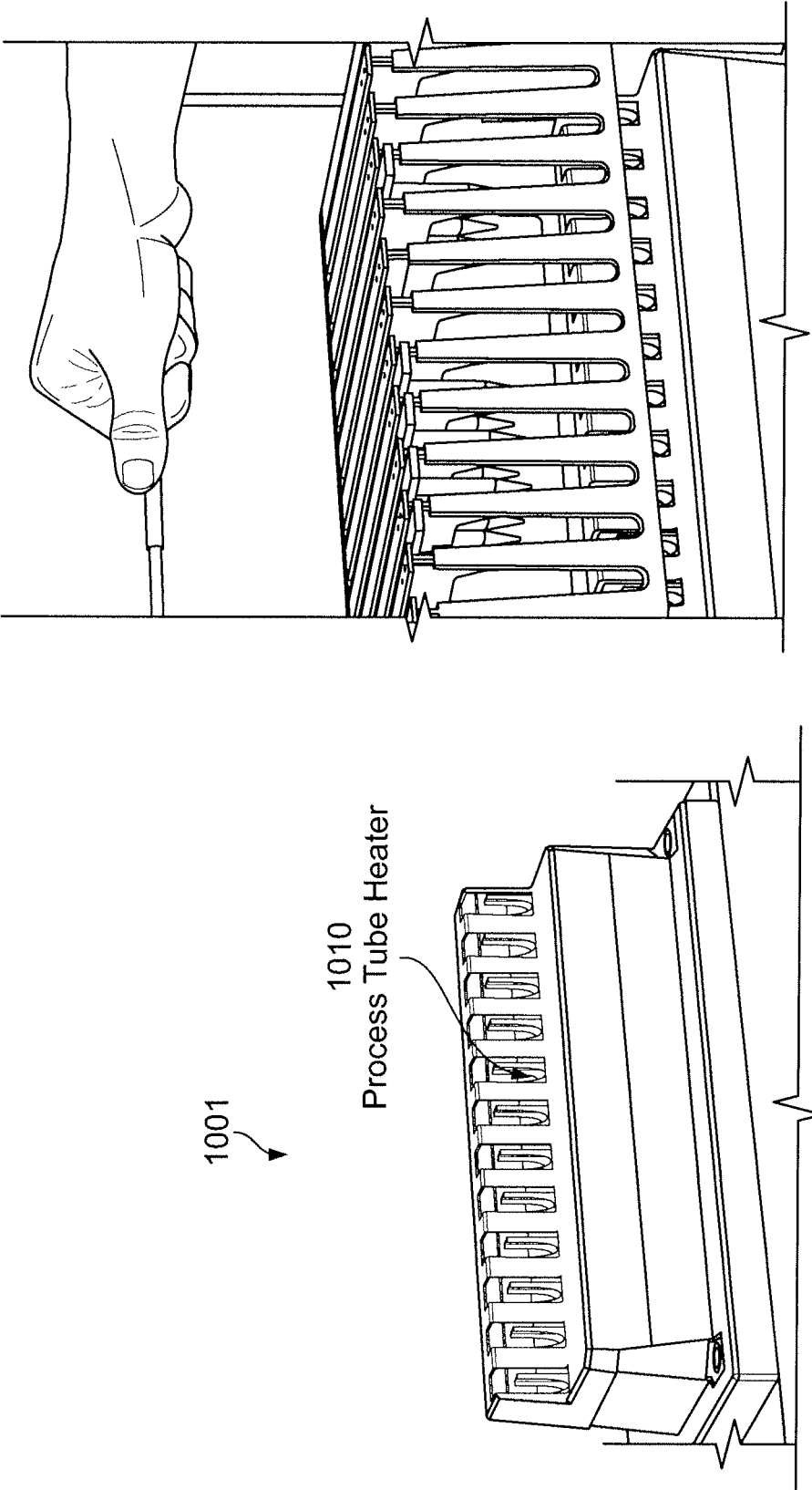
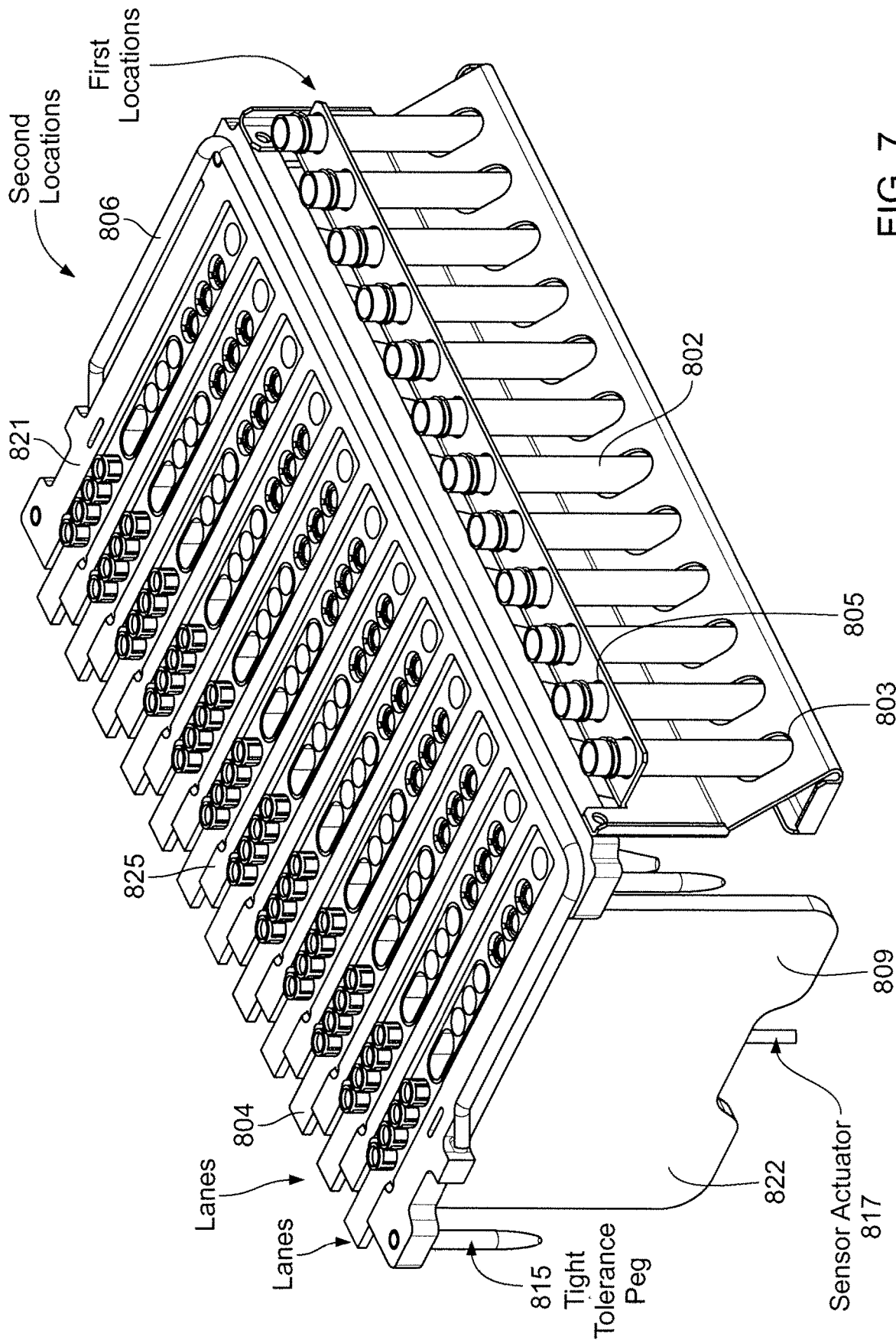


FIG. 6



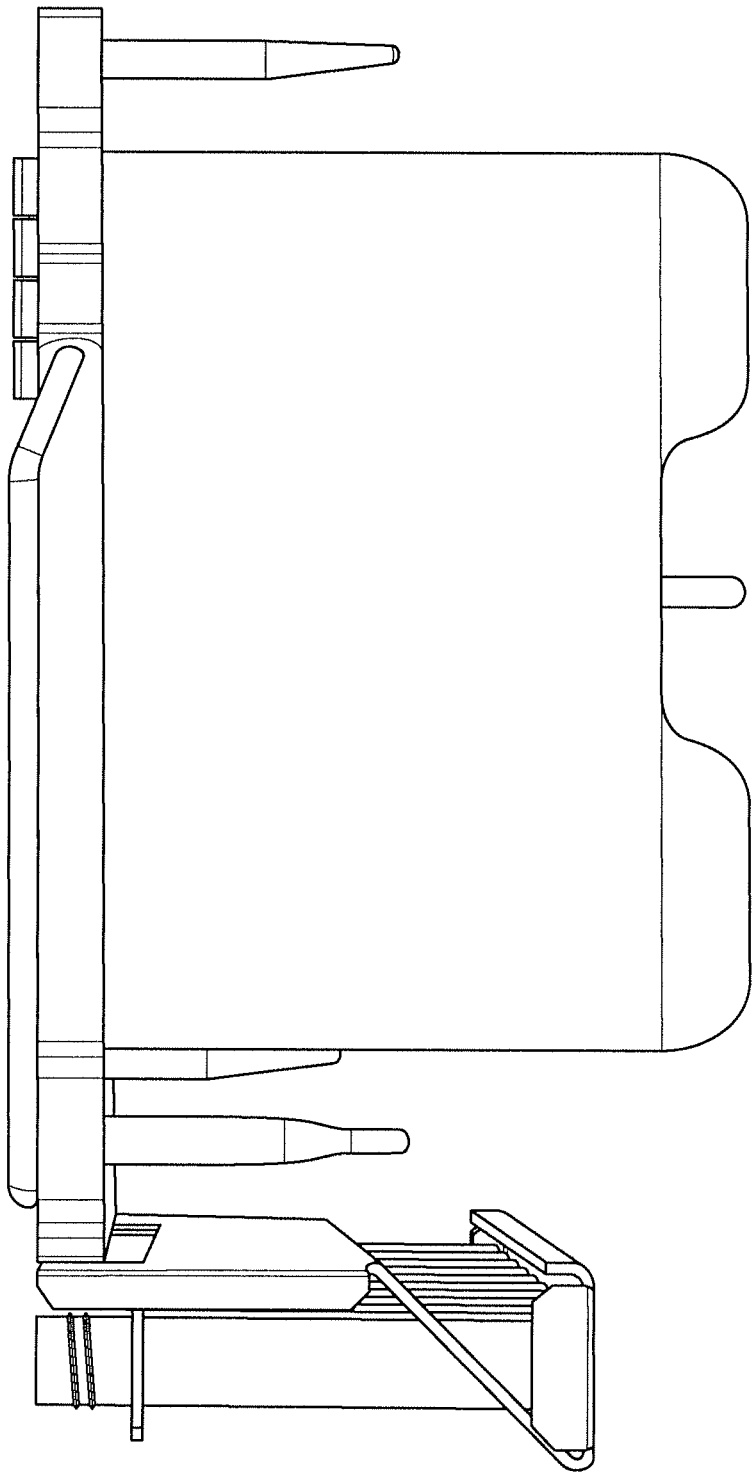


FIG. 8A

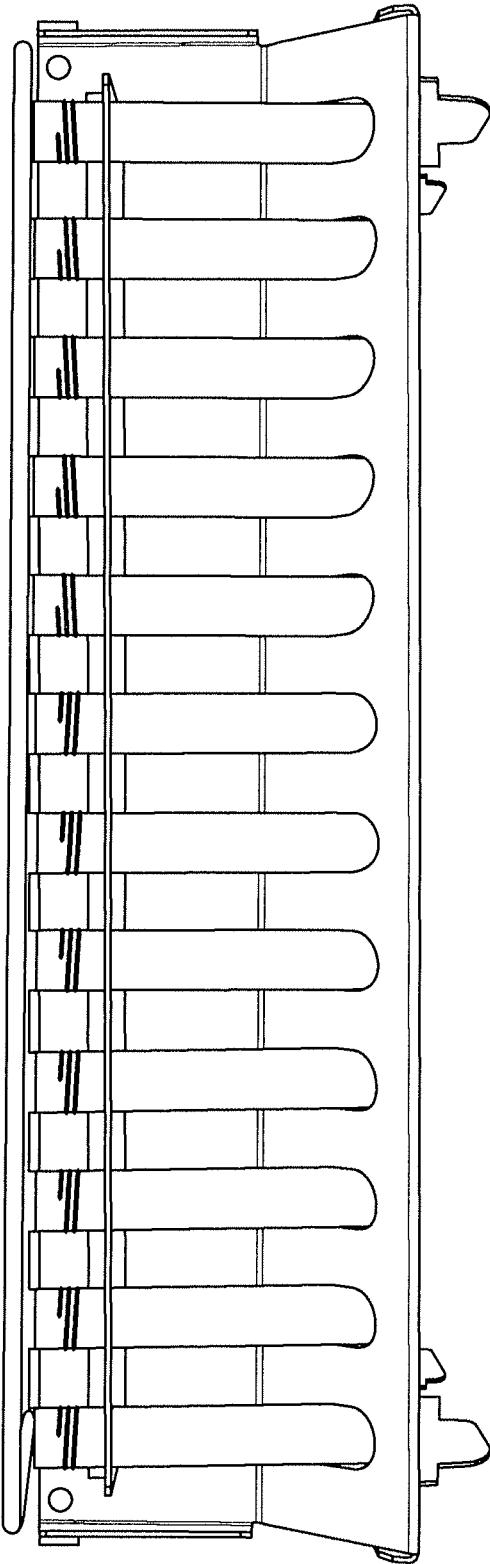


FIG. 8B

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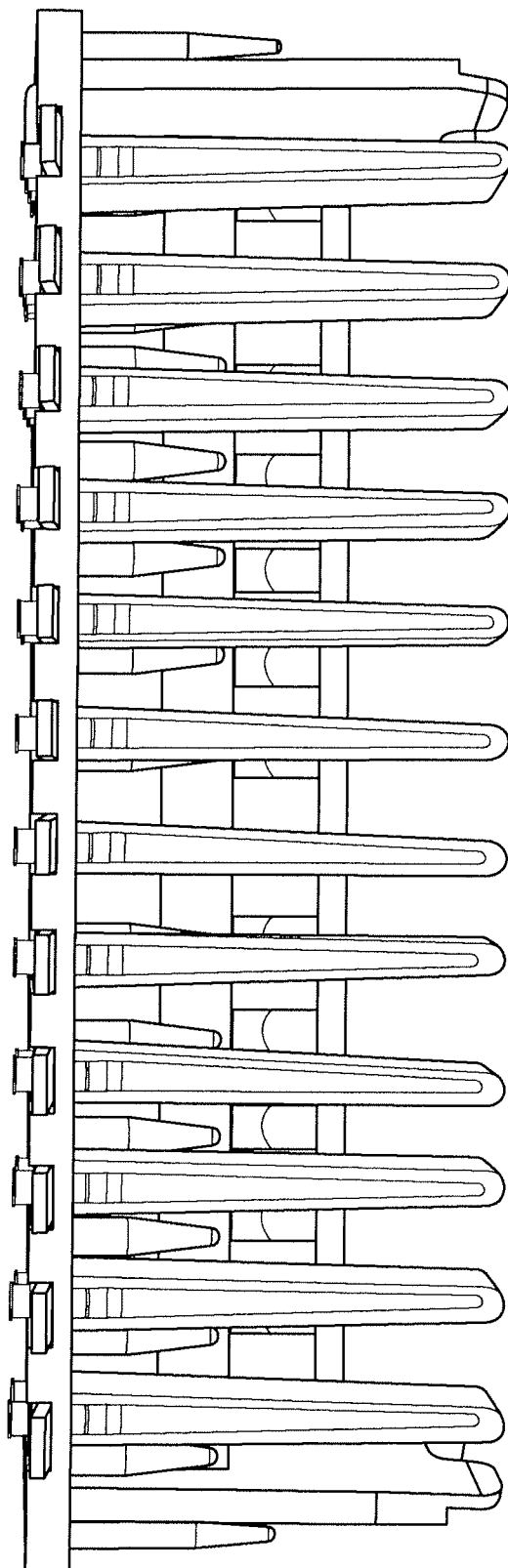


FIG. 8C

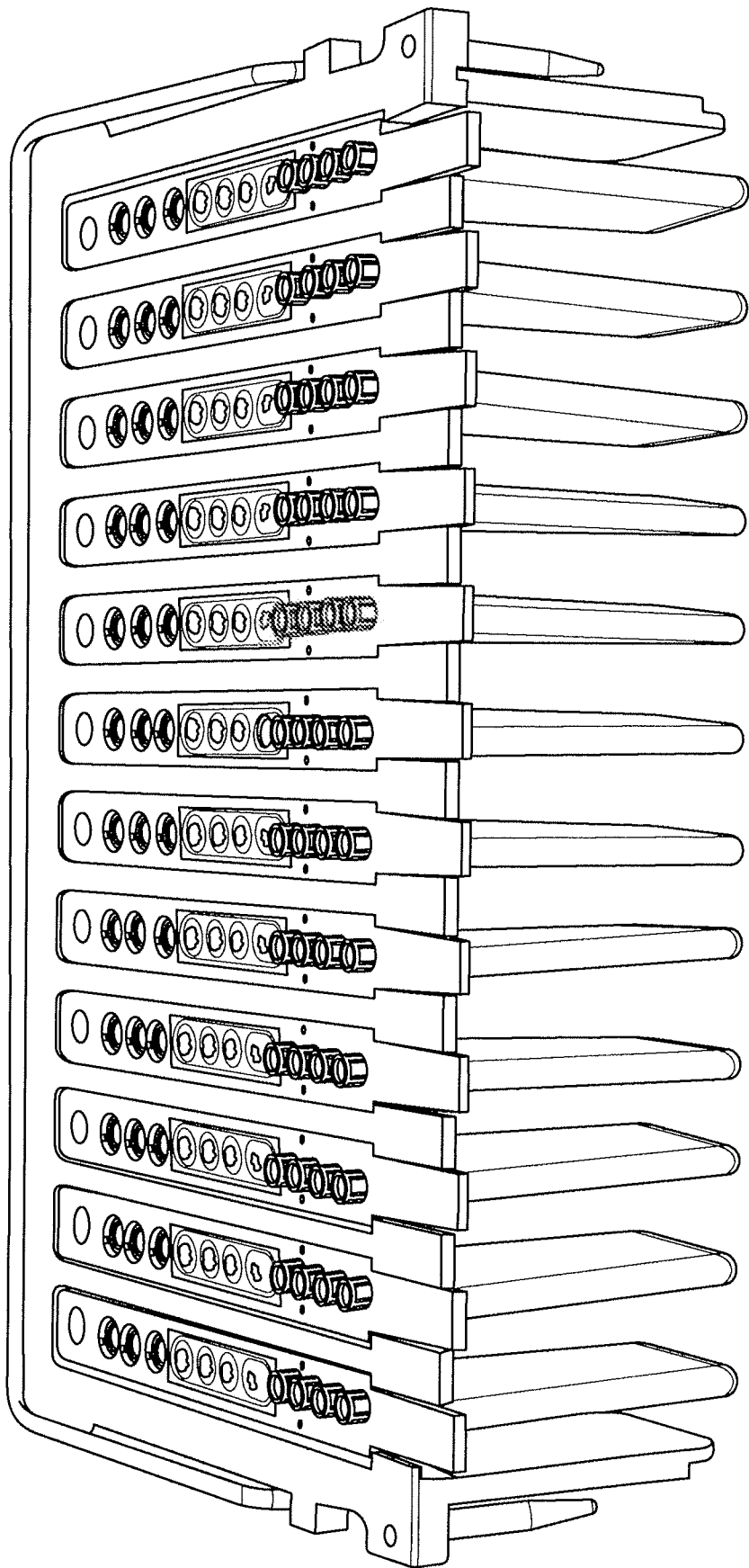


FIG. 8D

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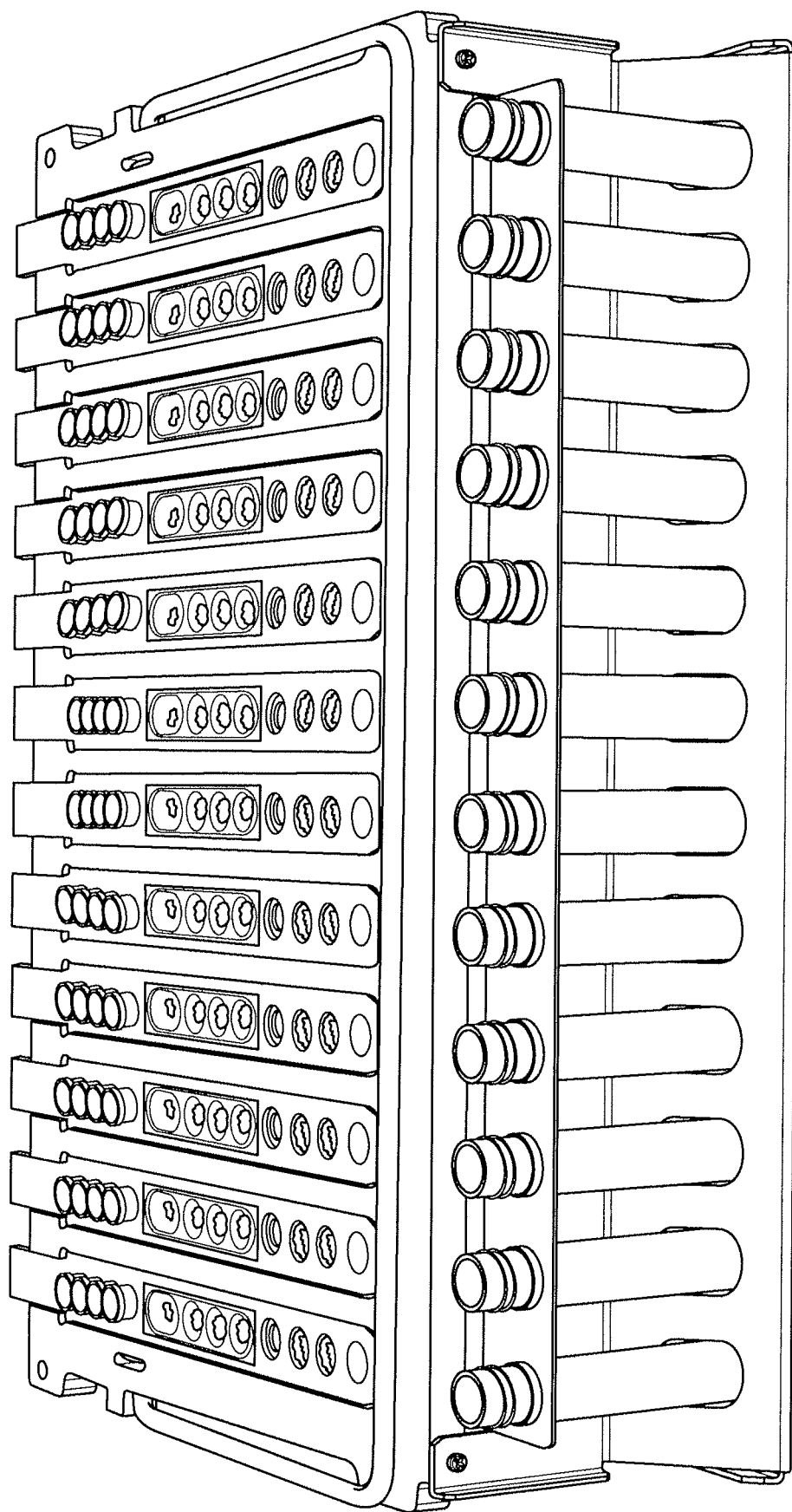


FIG. 8E

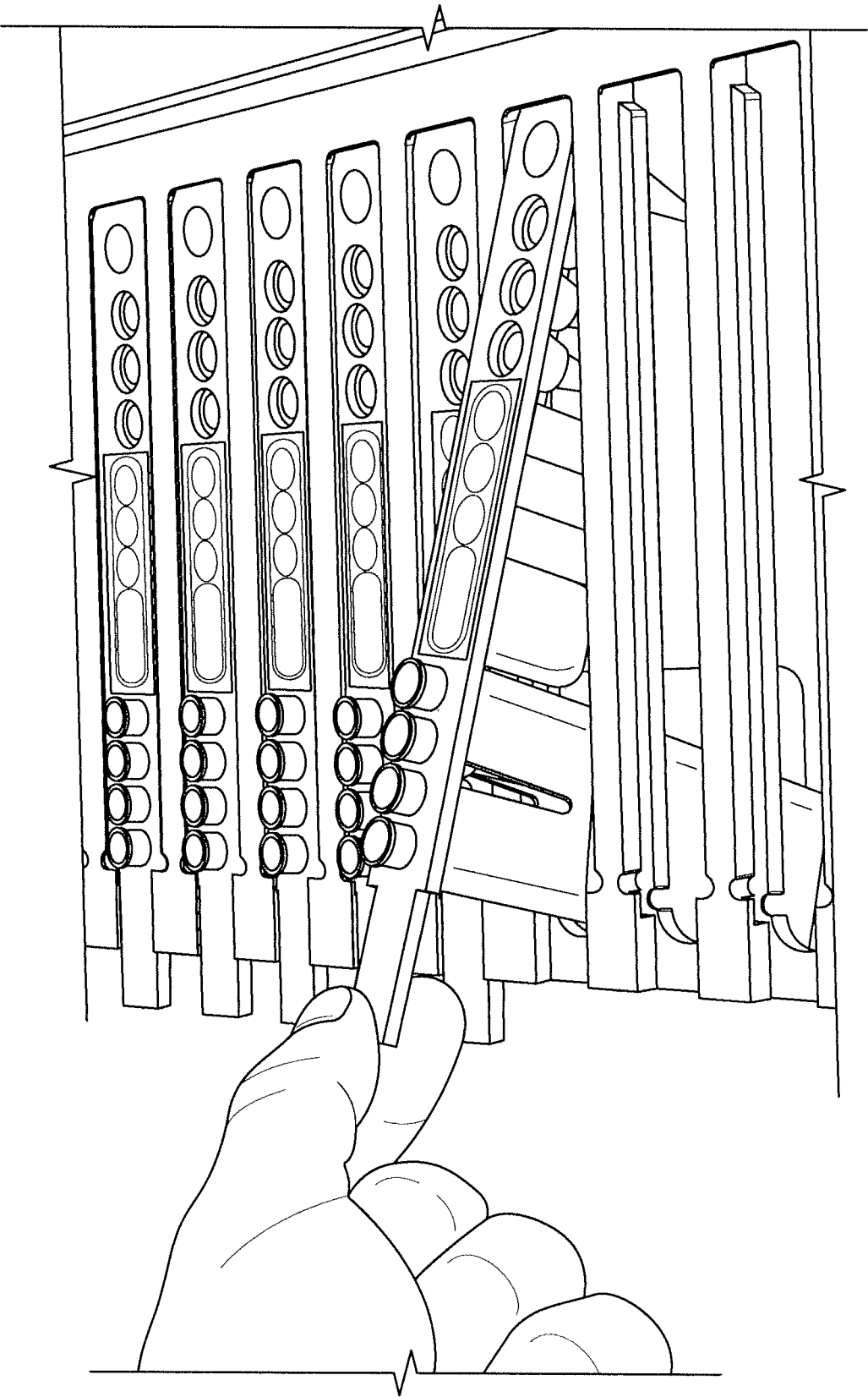


FIG. 8F

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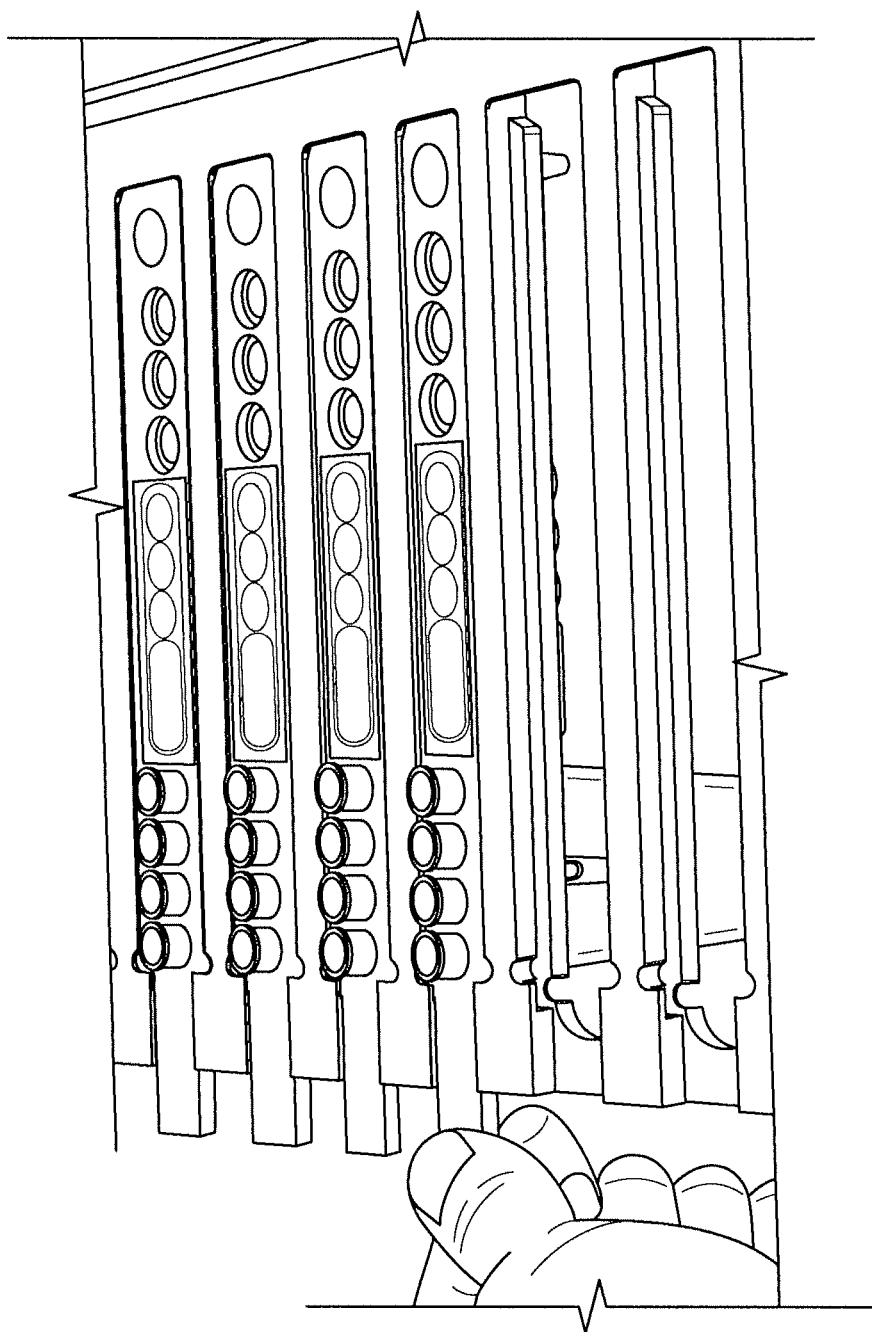


FIG. 8G

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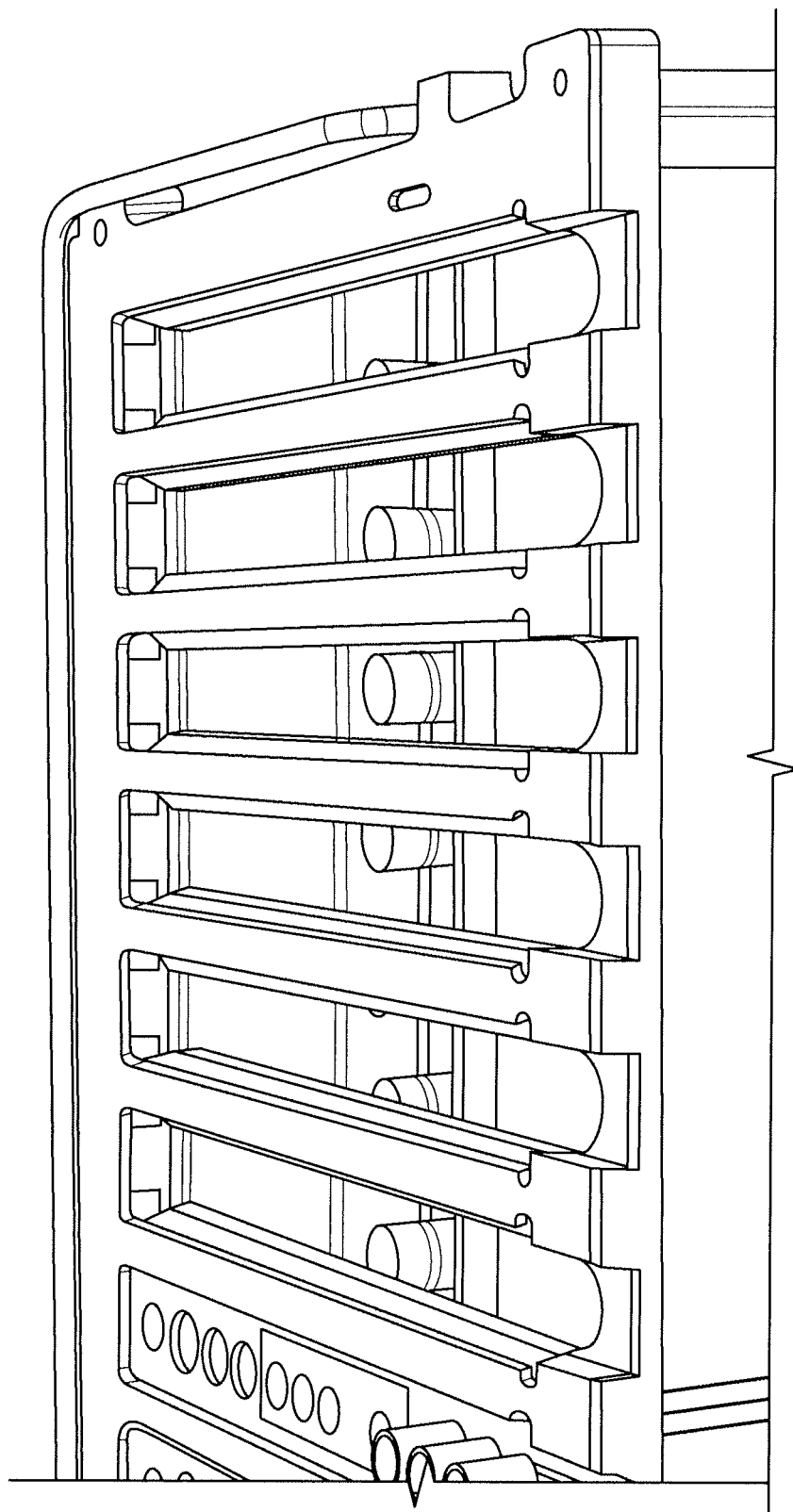


FIG. 8H

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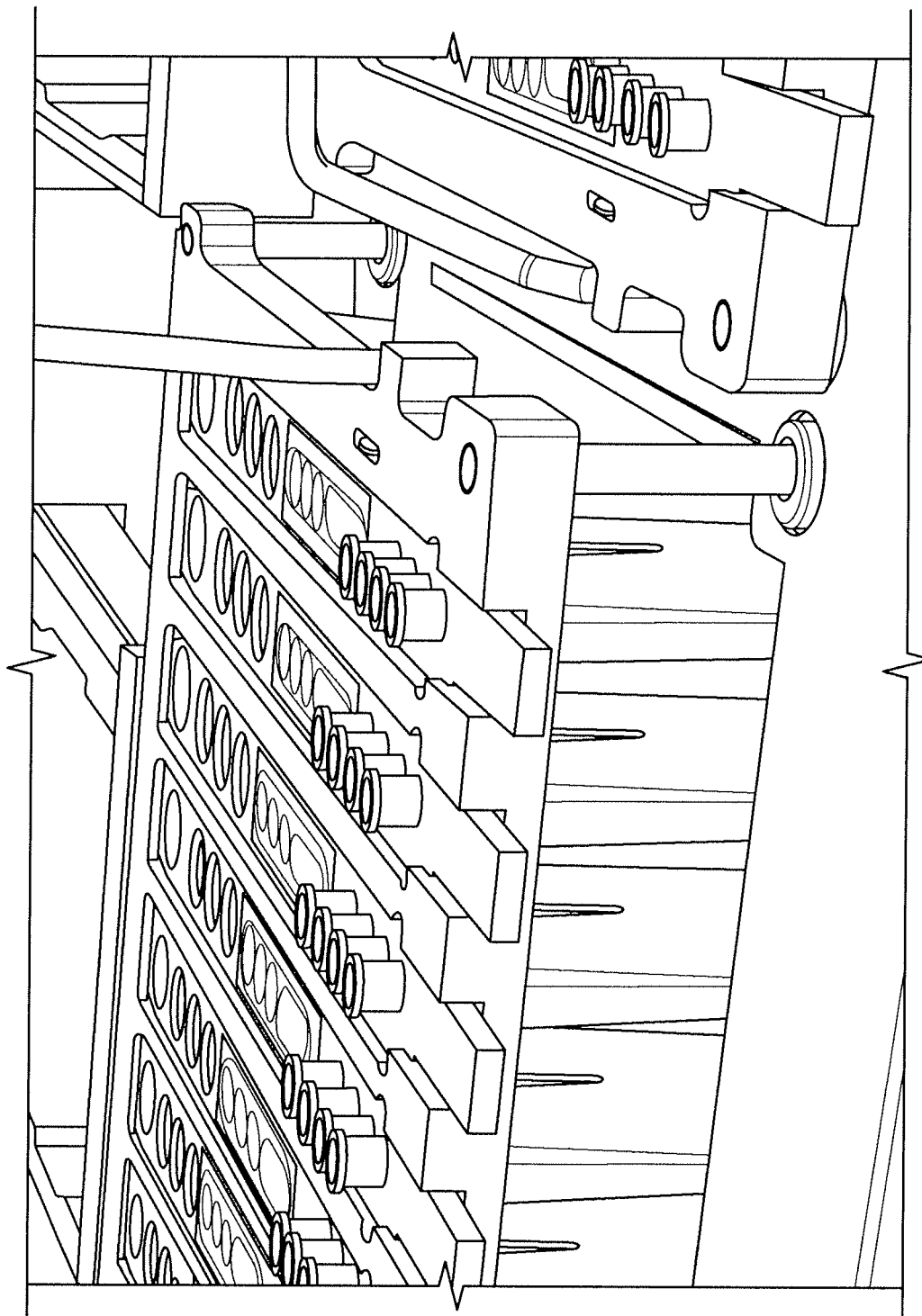


FIG. 8I

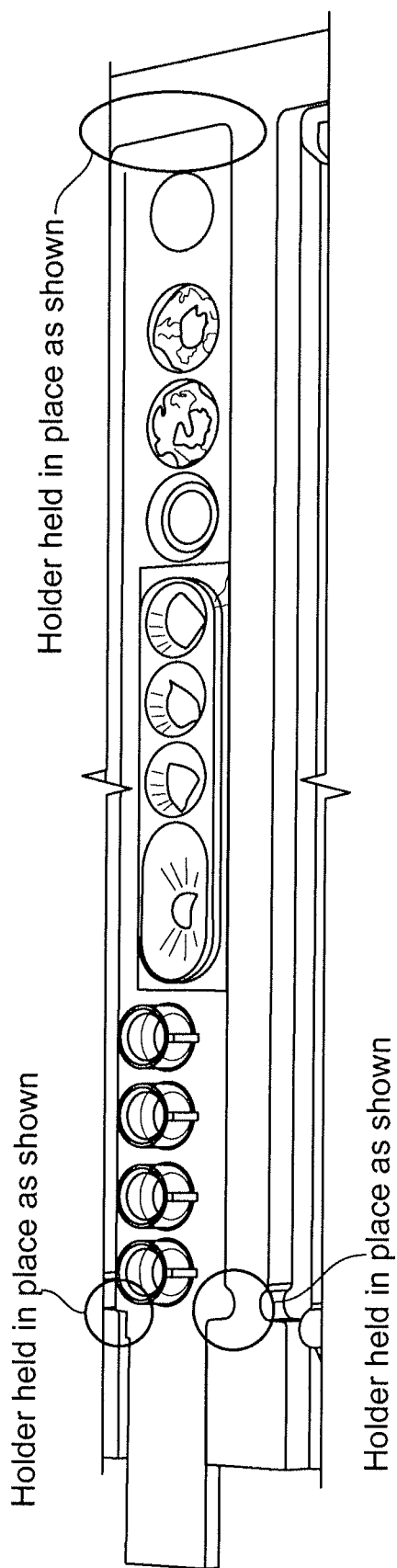


Fig. 8J

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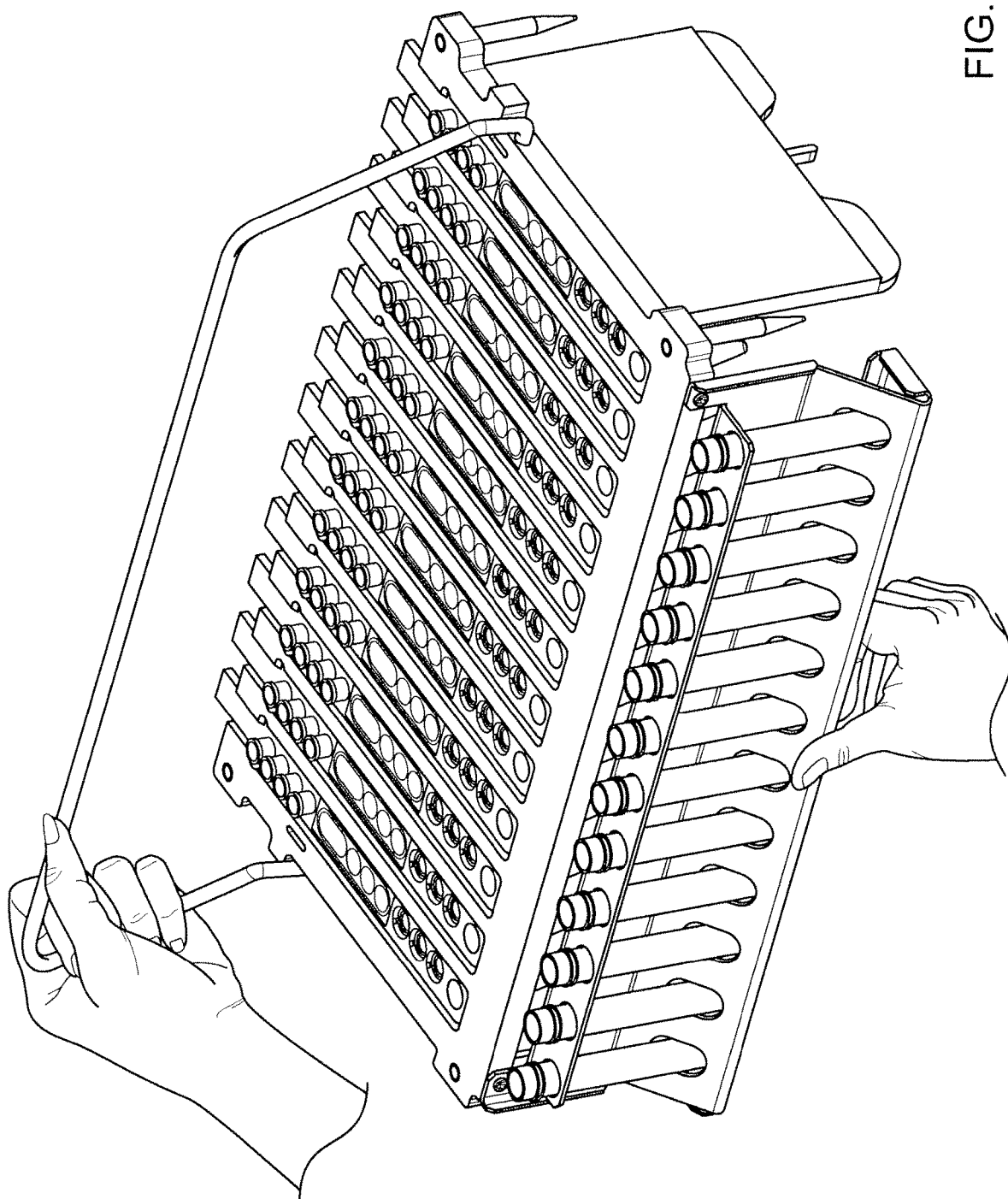


FIG. 8K

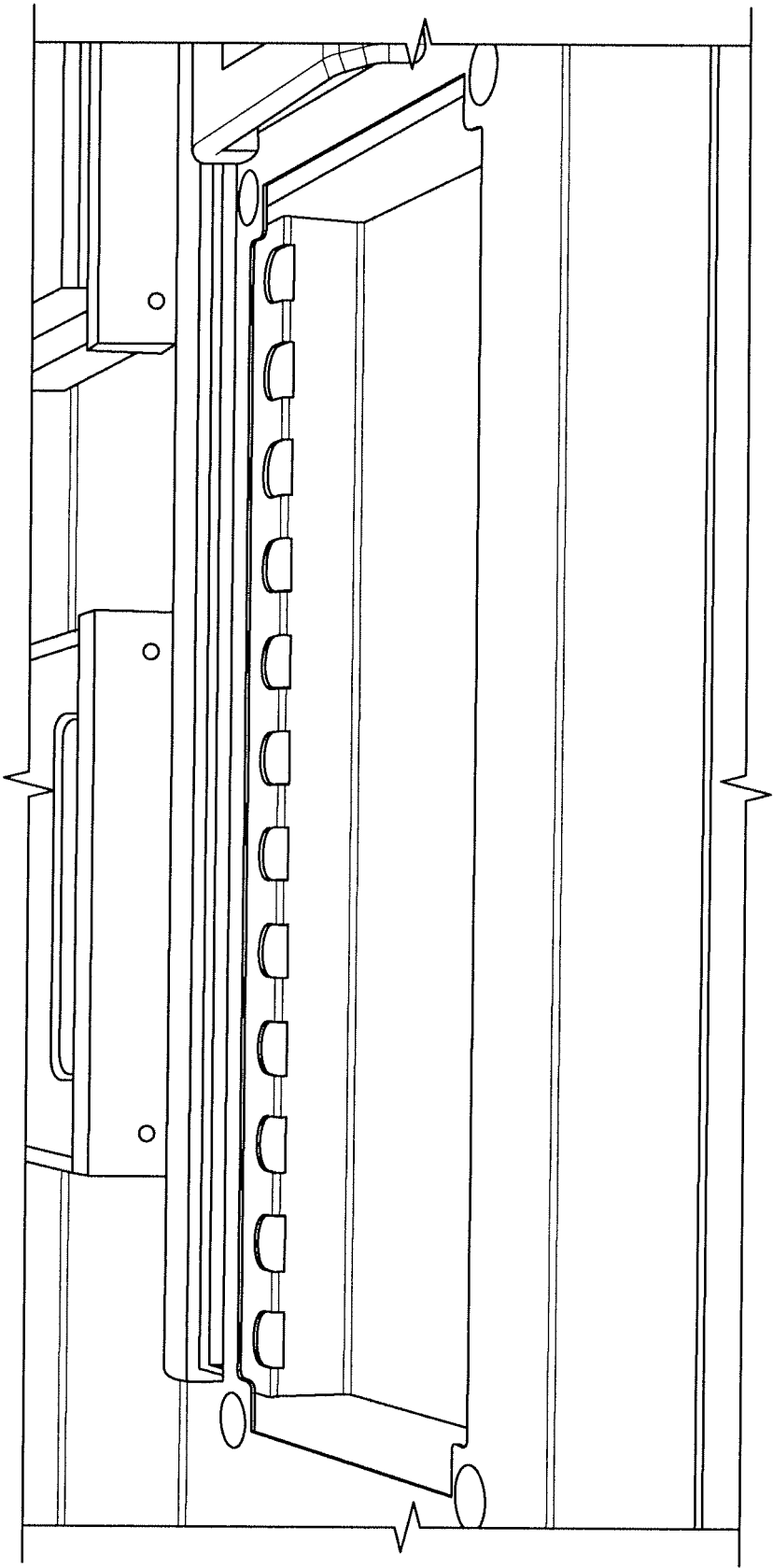
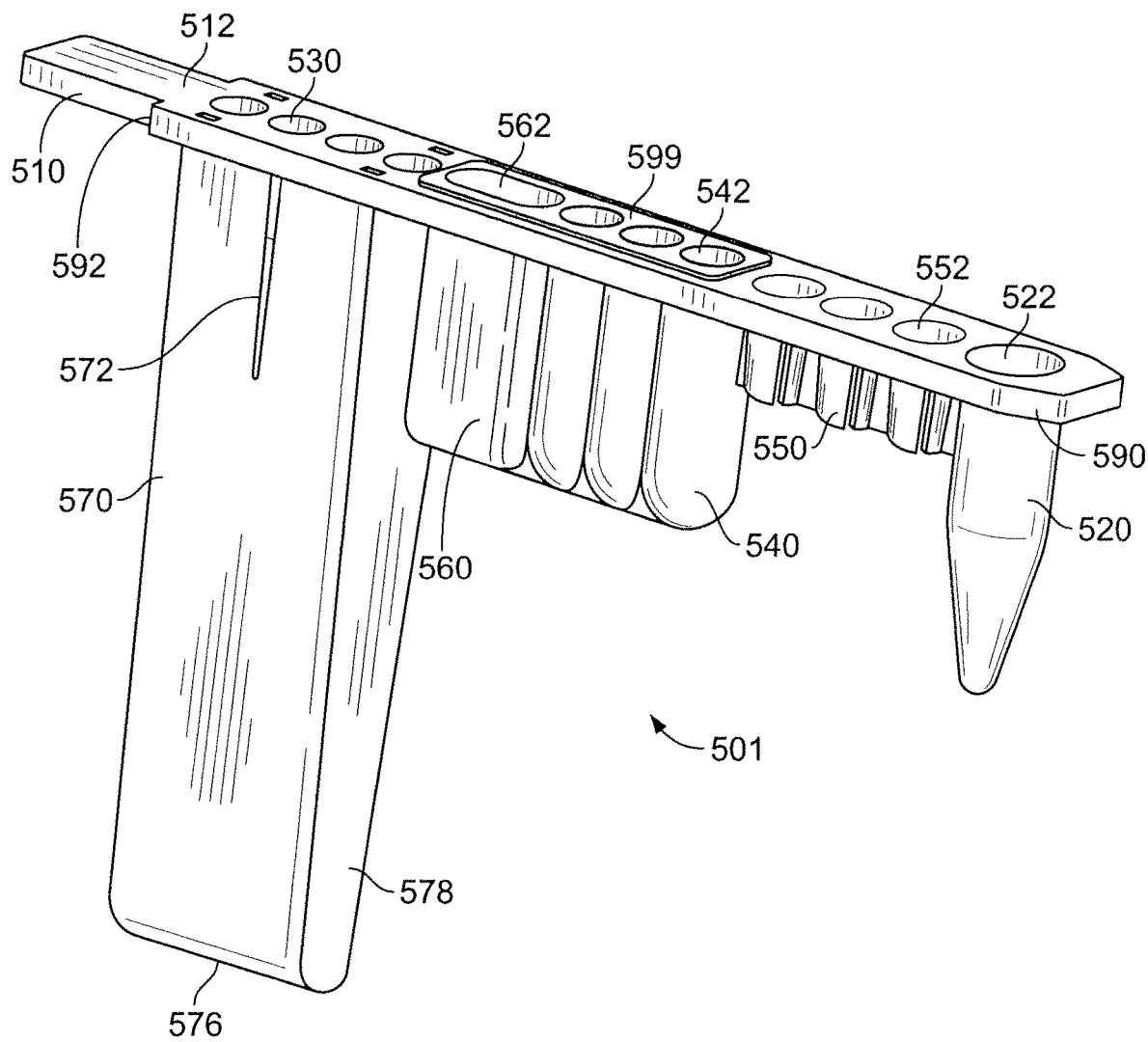


FIG. 9



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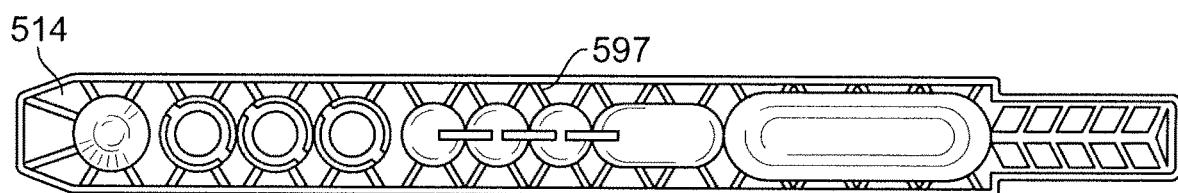


FIG. 10B

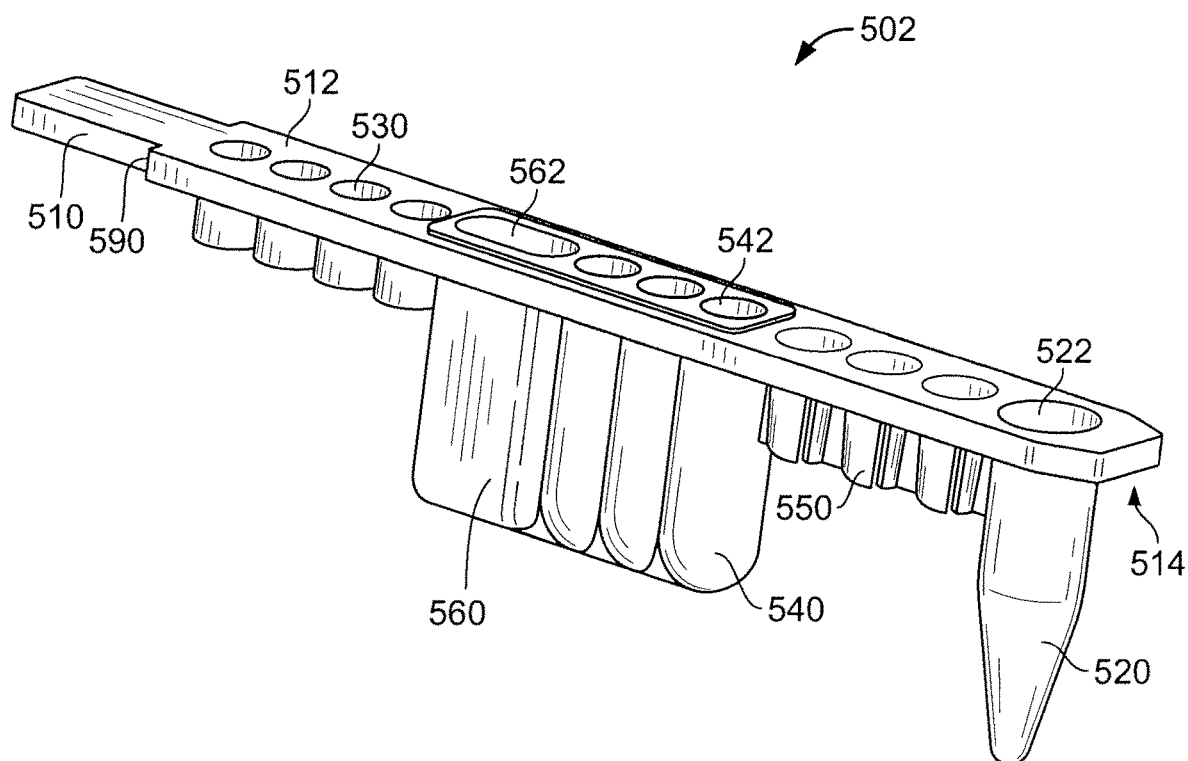


FIG. 11

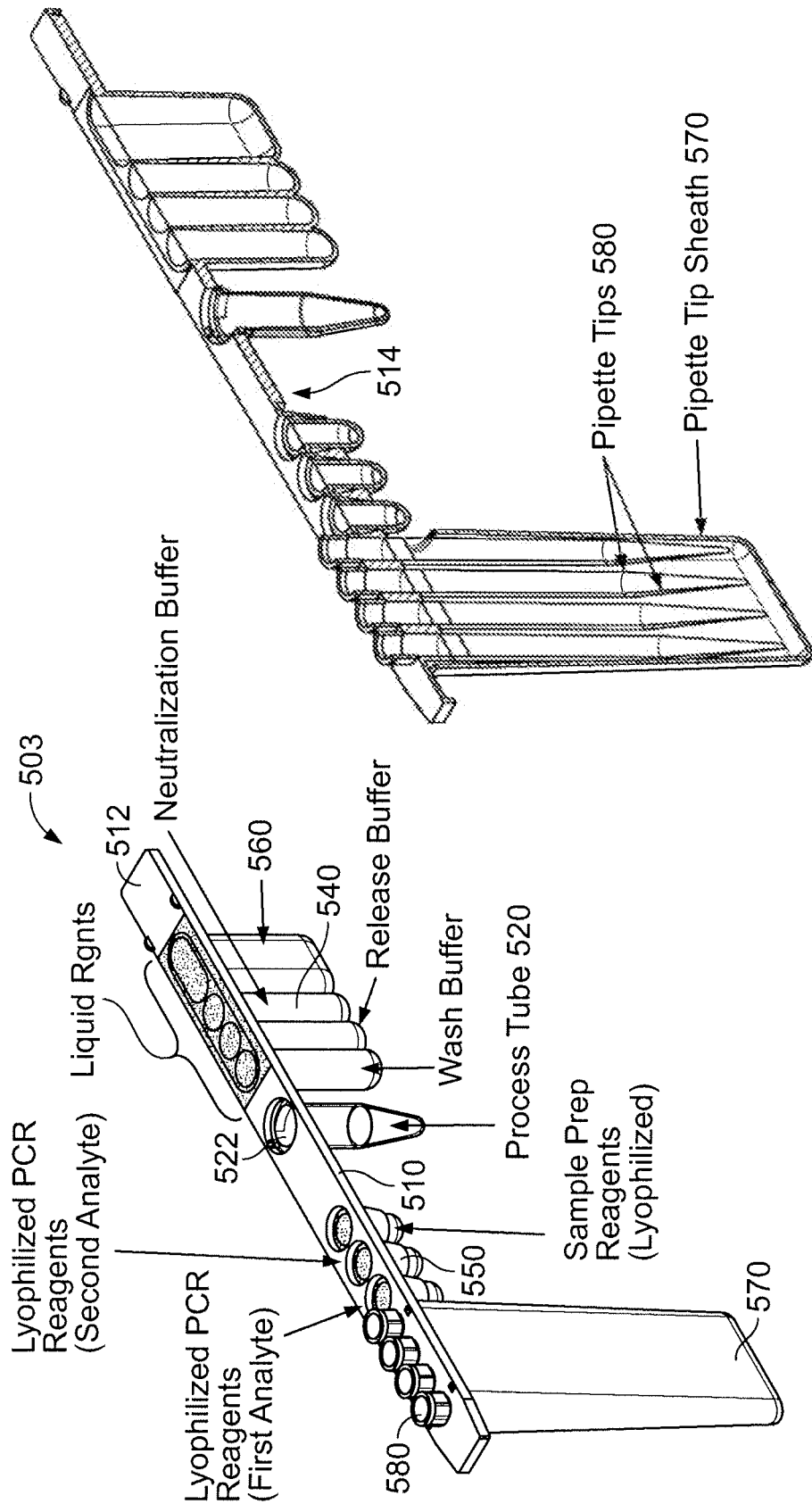


FIG. 12B

FIG. 12A

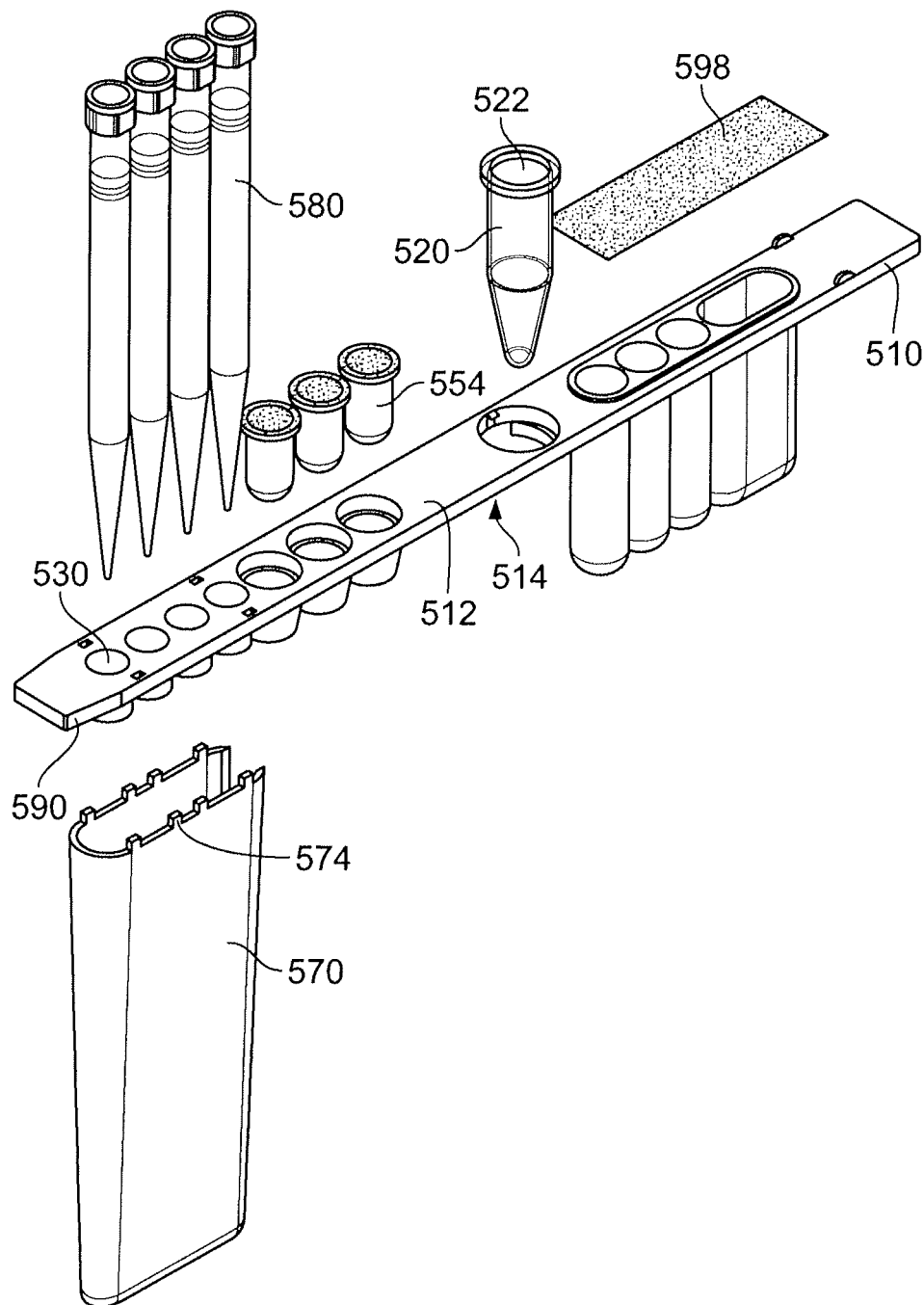


FIG. 12C

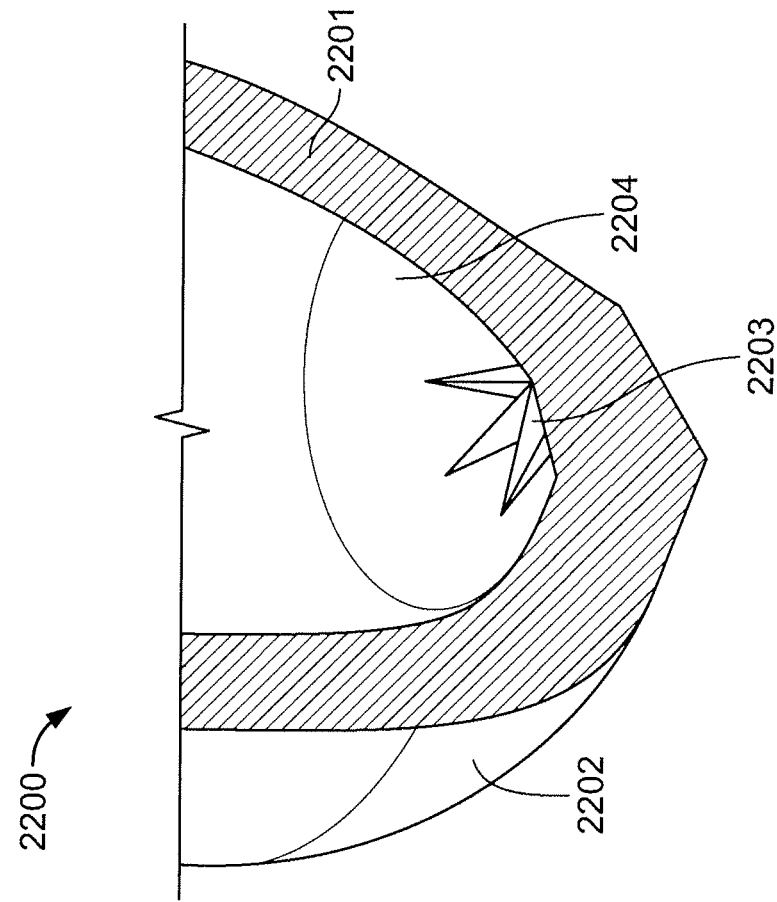


FIG. 13A

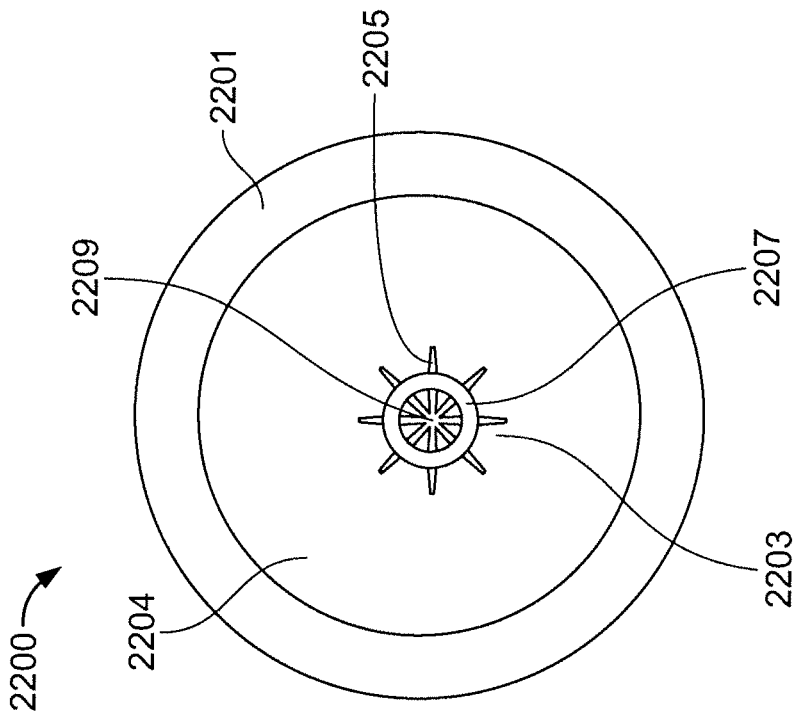


FIG. 13B

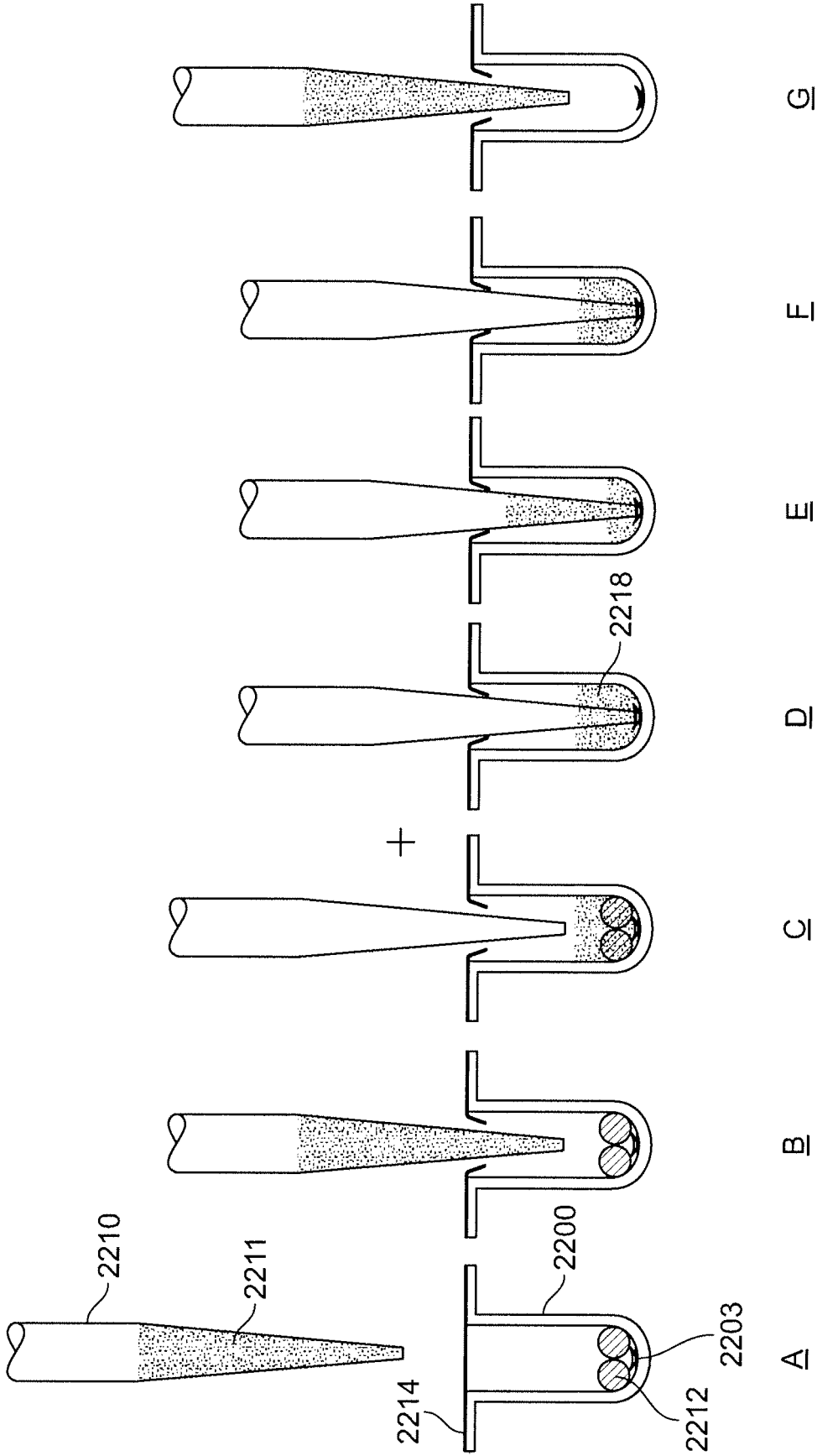
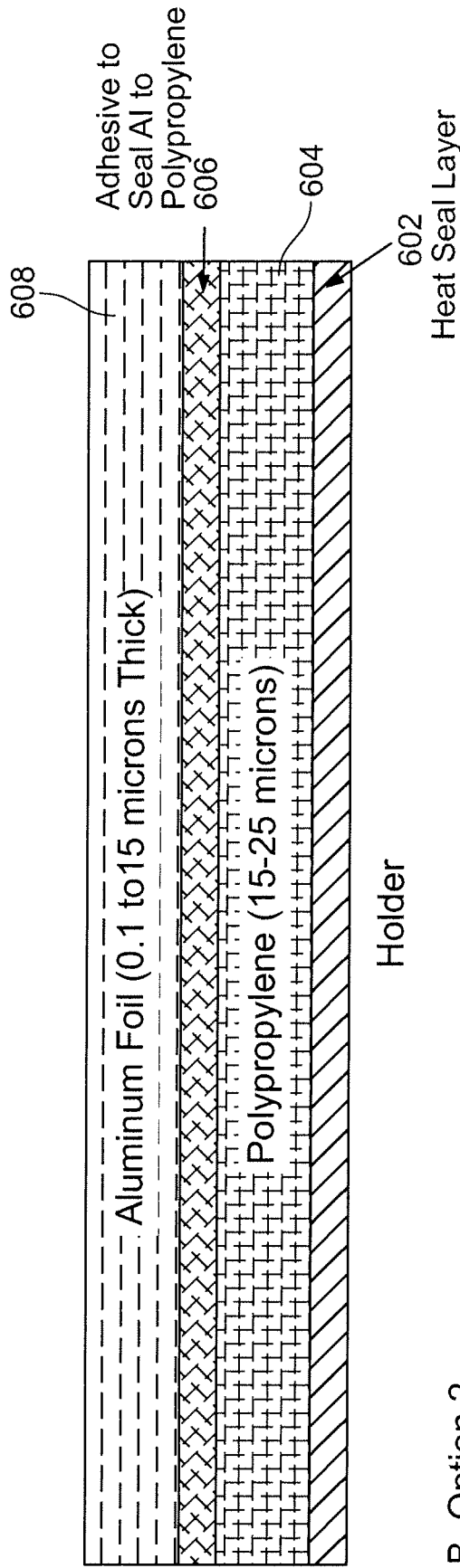


FIG. 14

A. Option 1



B. Option 2

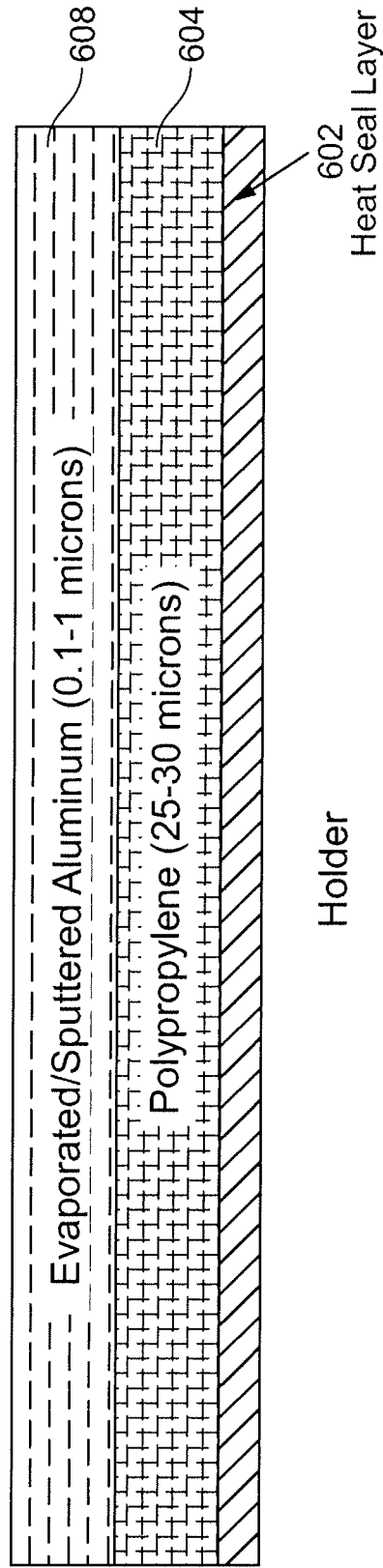


FIG. 15

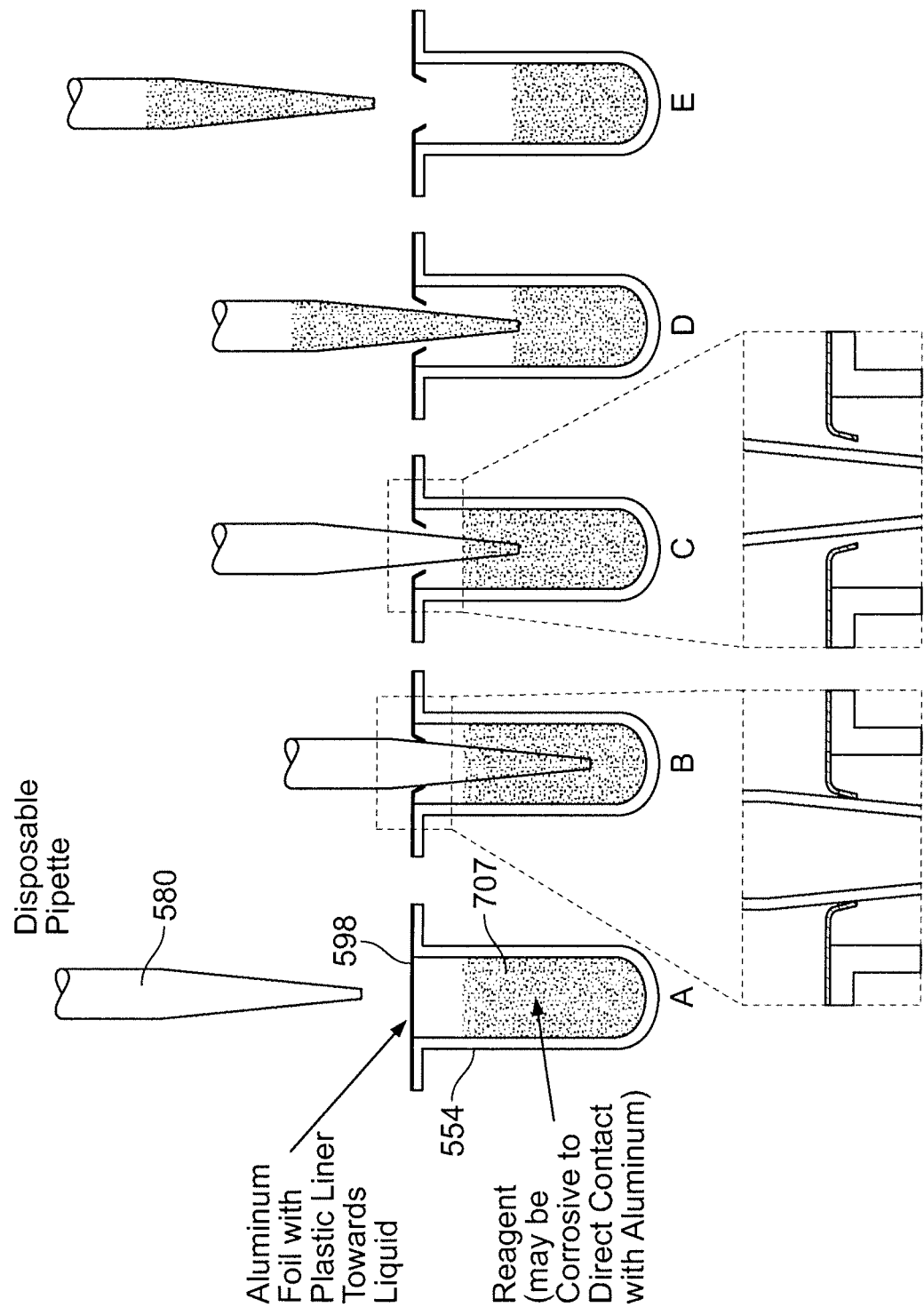


FIG. 16

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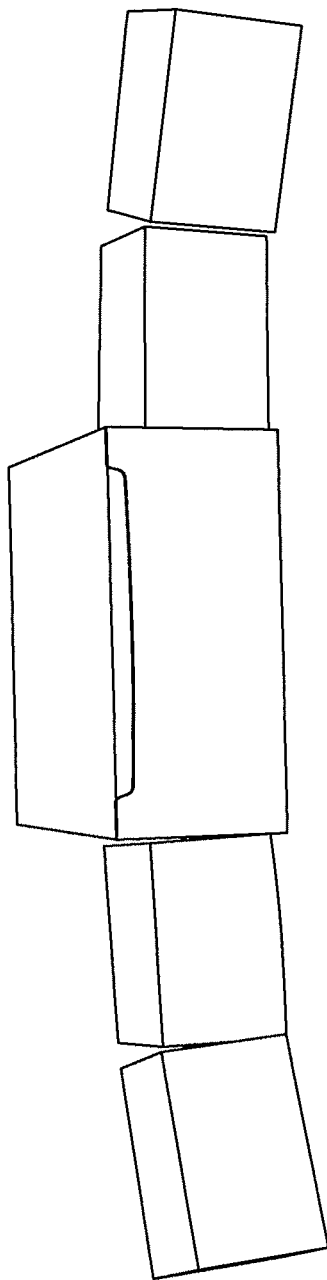


FIG. 17A

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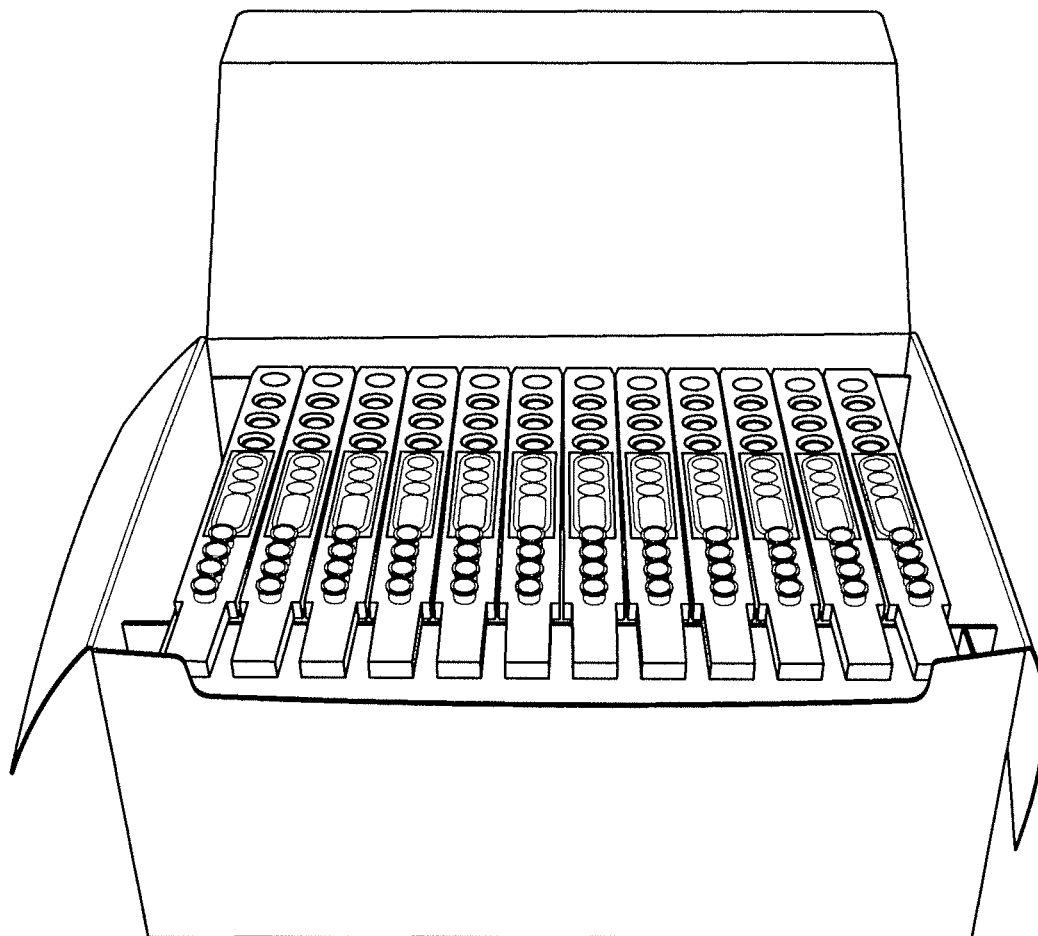


FIG. 17B

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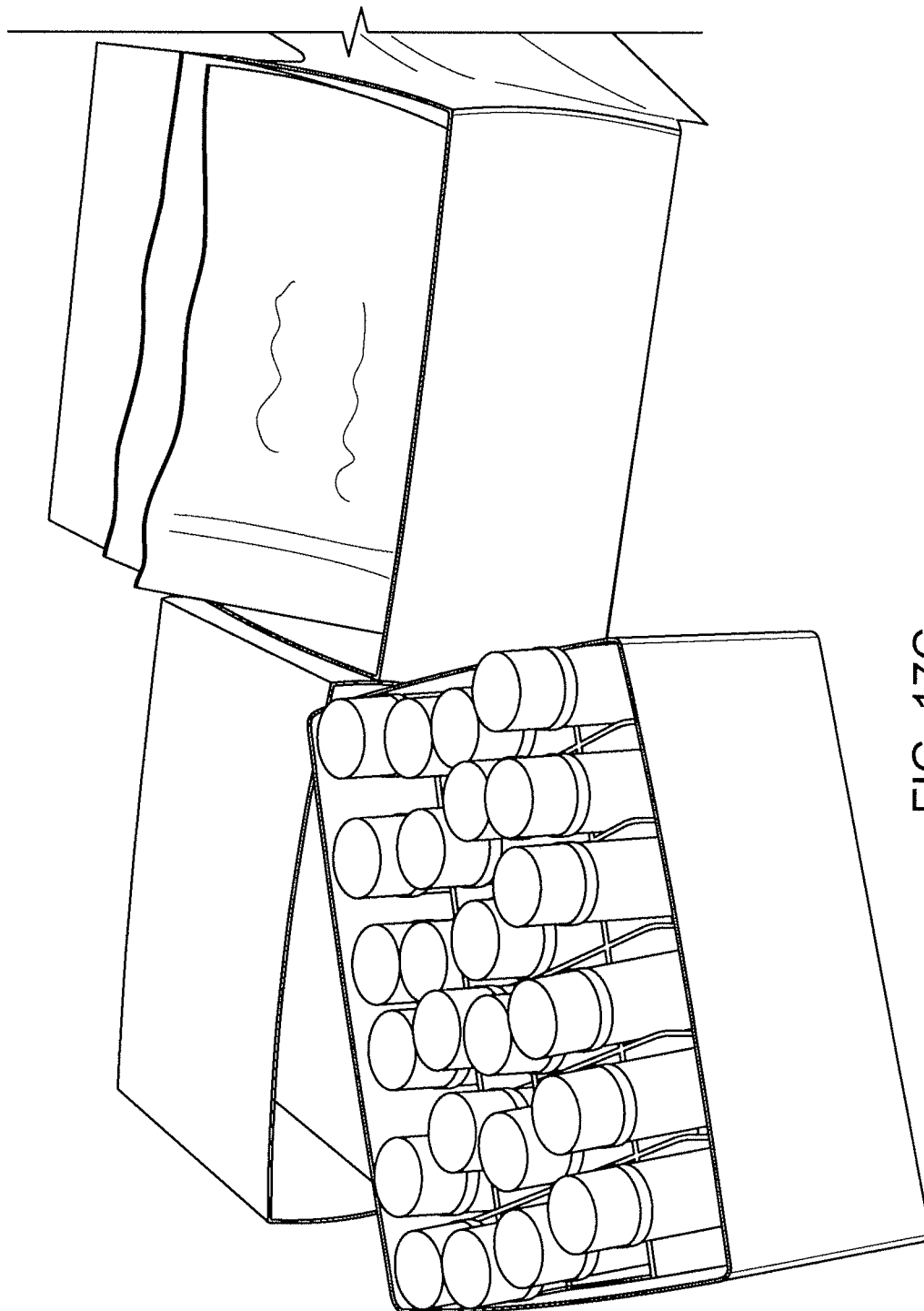


FIG. 17C

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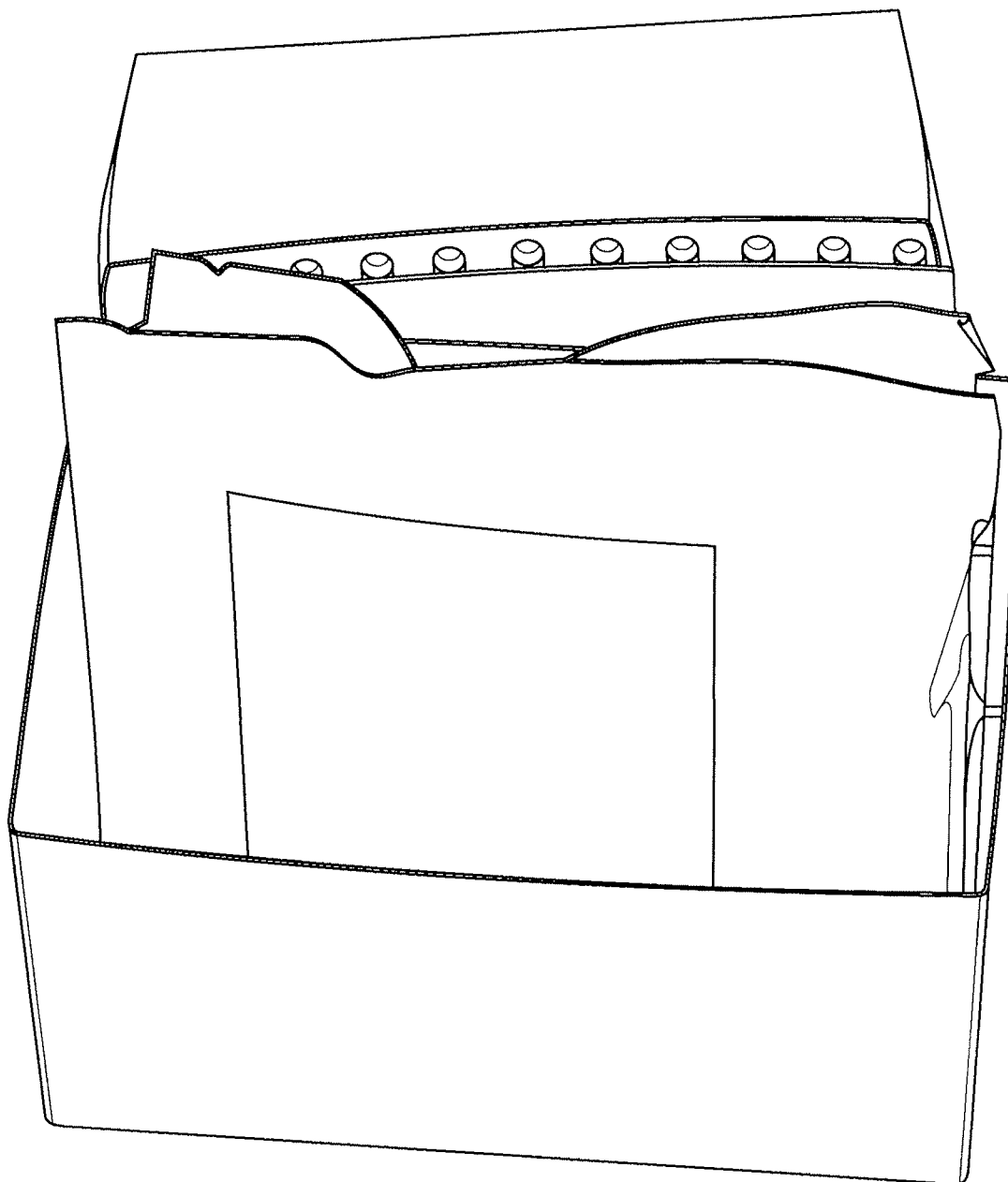
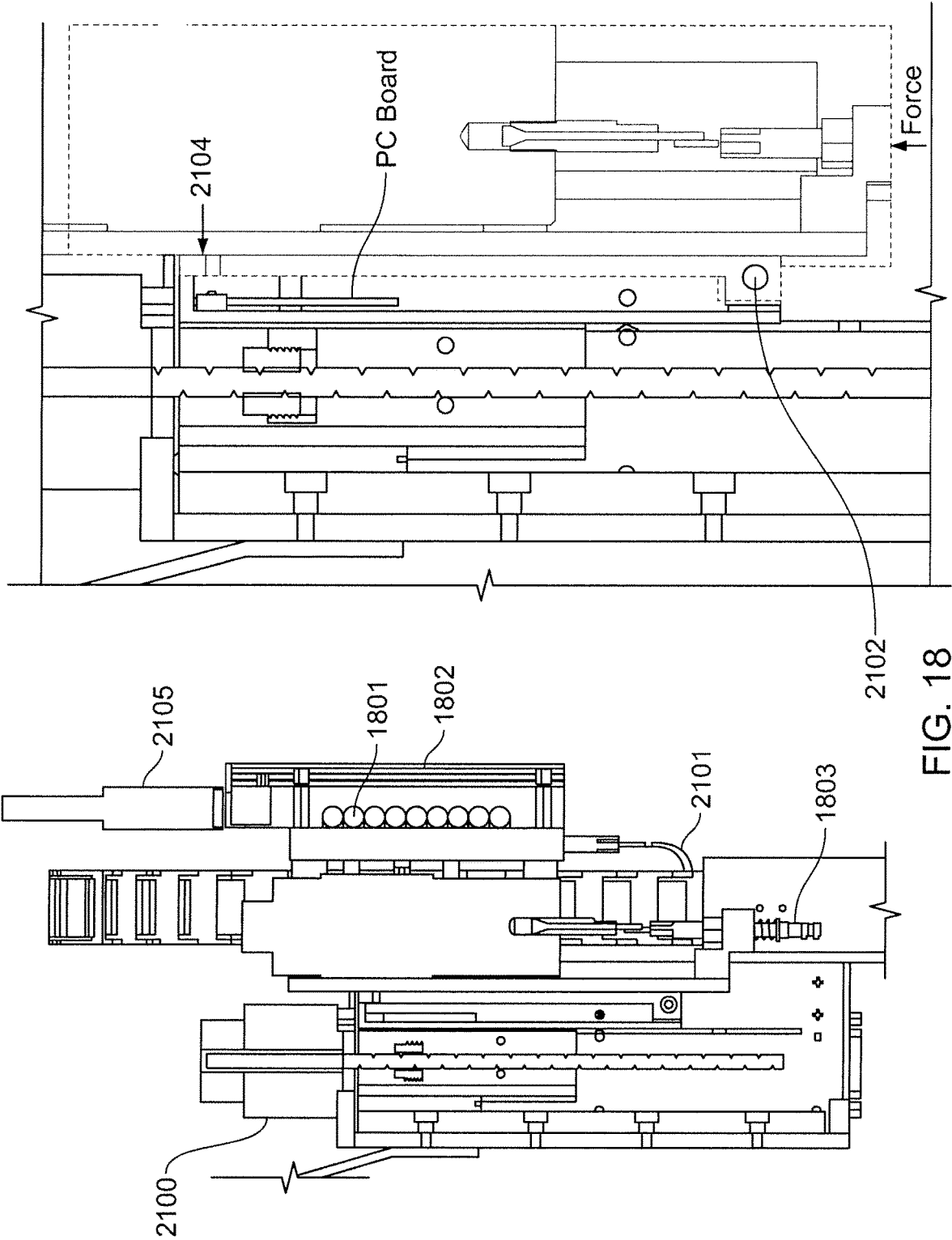


FIG. 17D



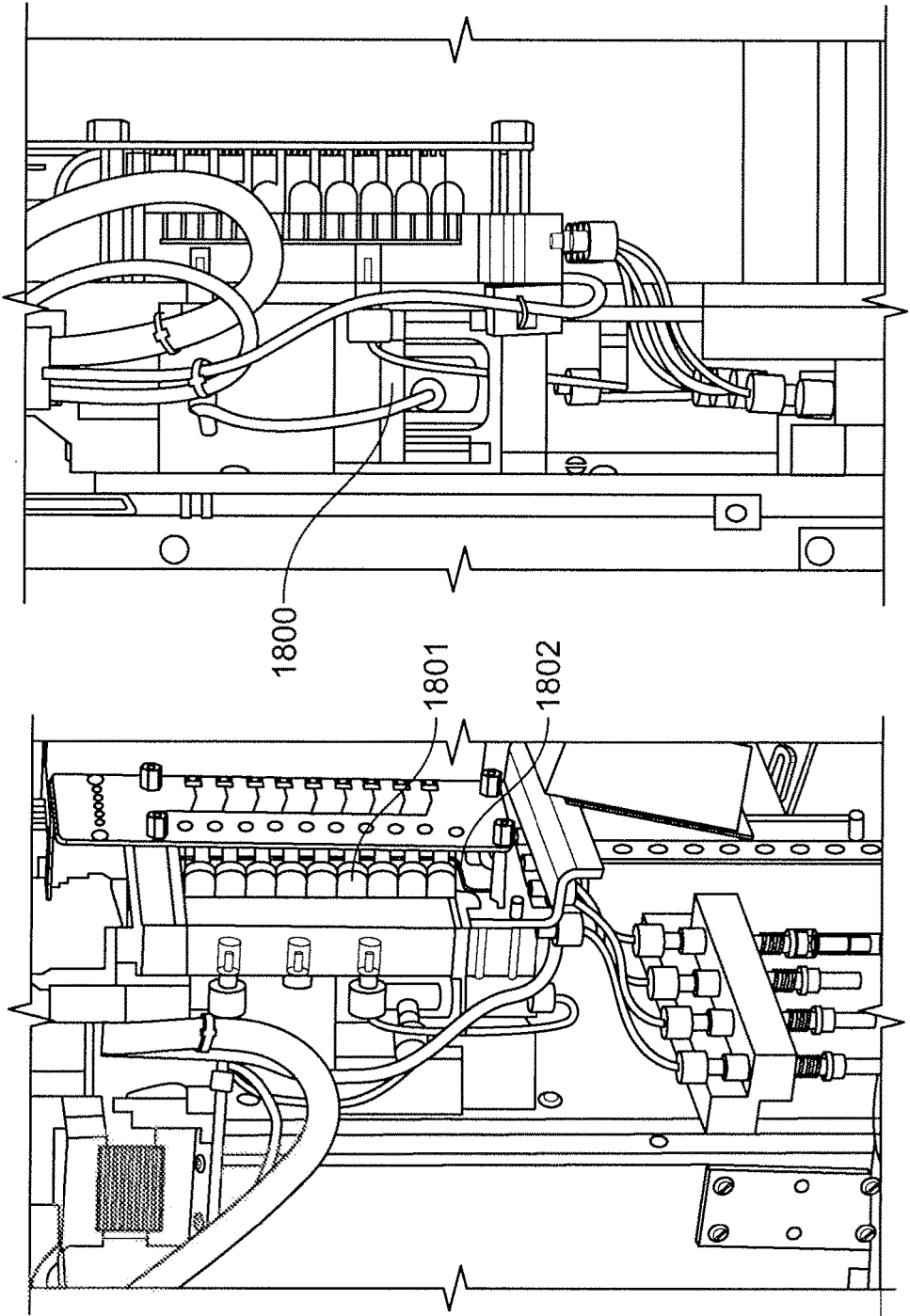


FIG. 19B

FIG. 19A

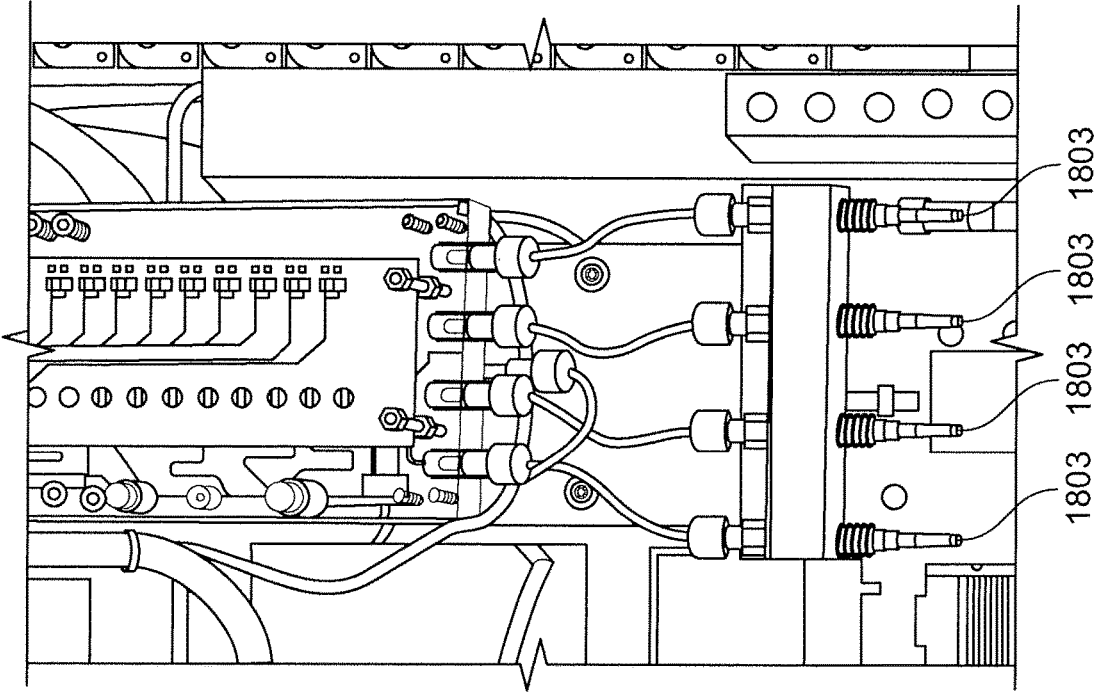


FIG. 19C

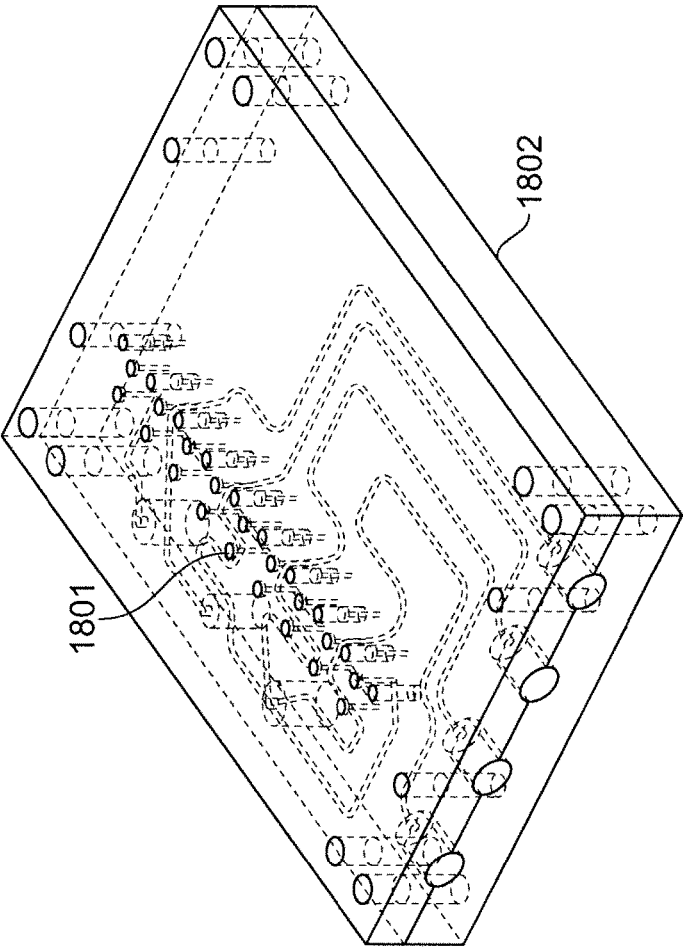
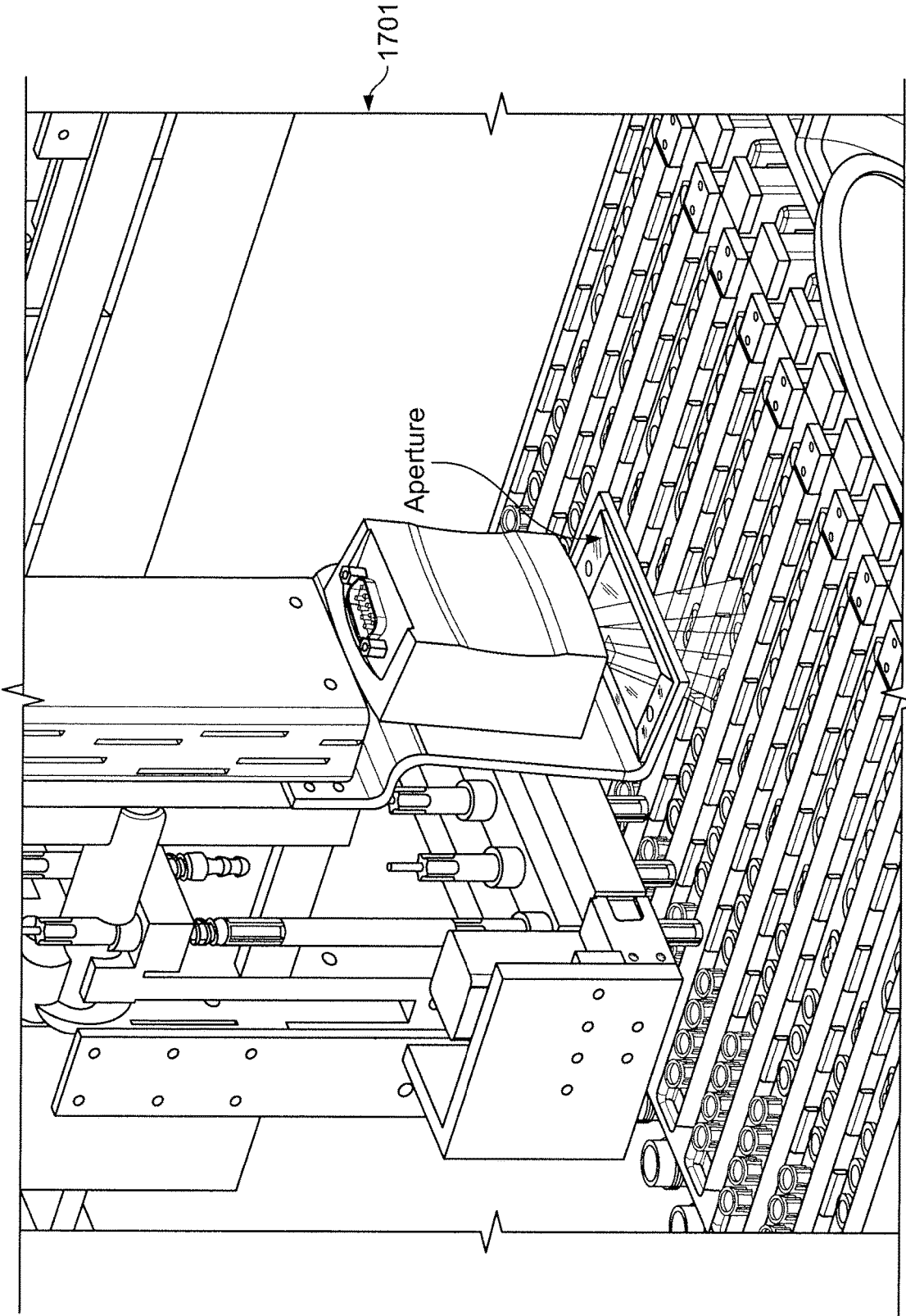


FIG. 20



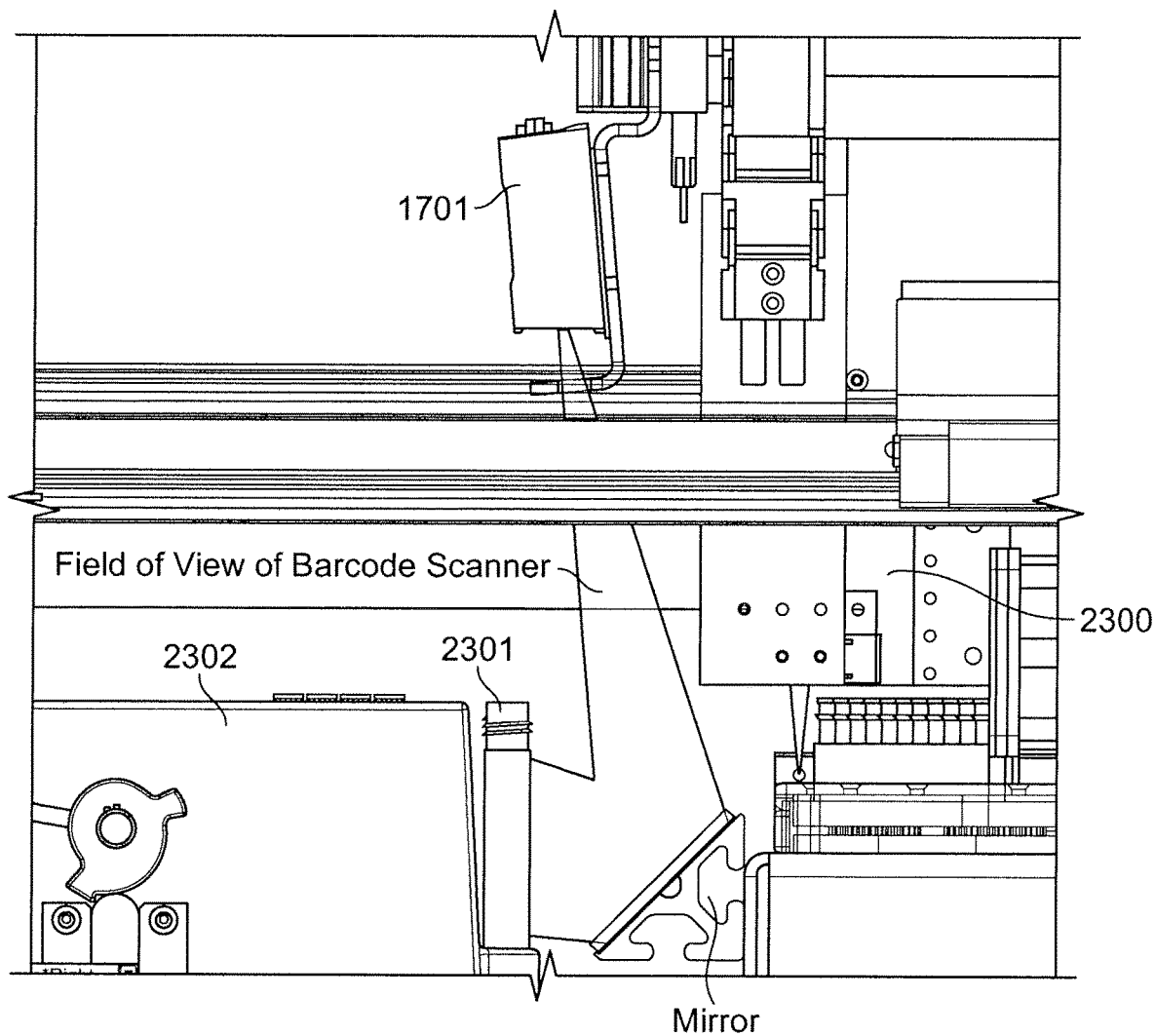


FIG. 22

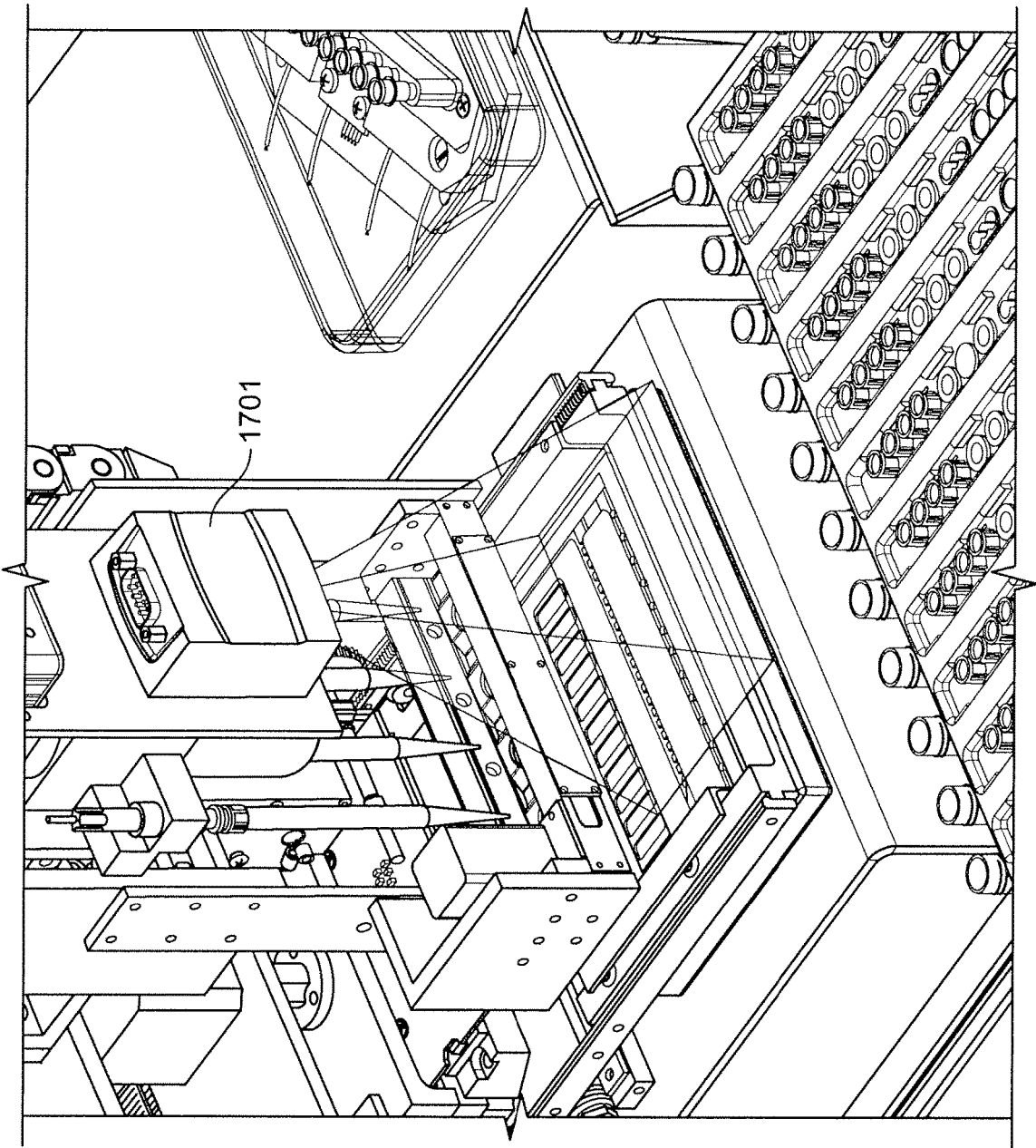


FIG. 23

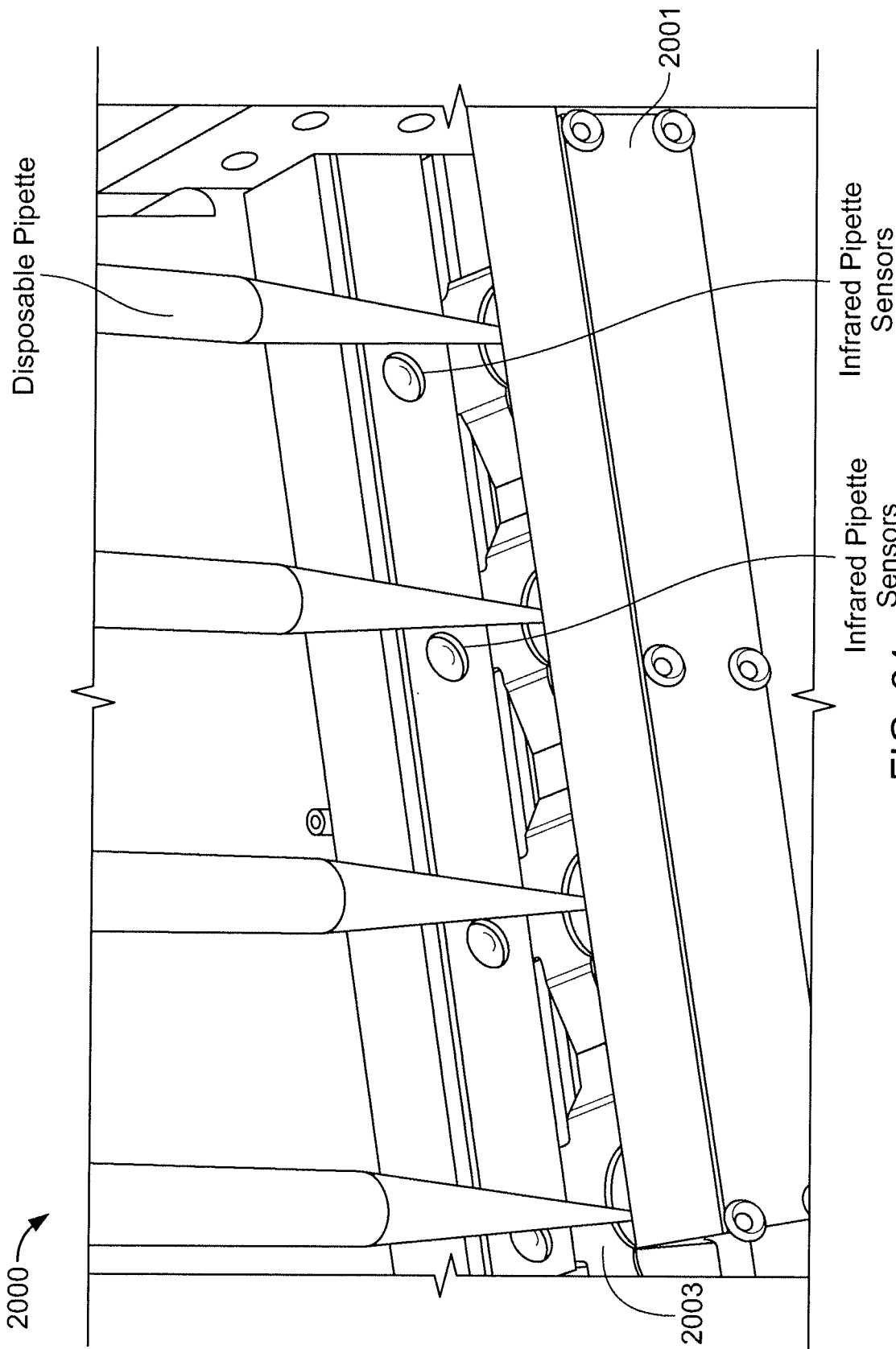


FIG. 24

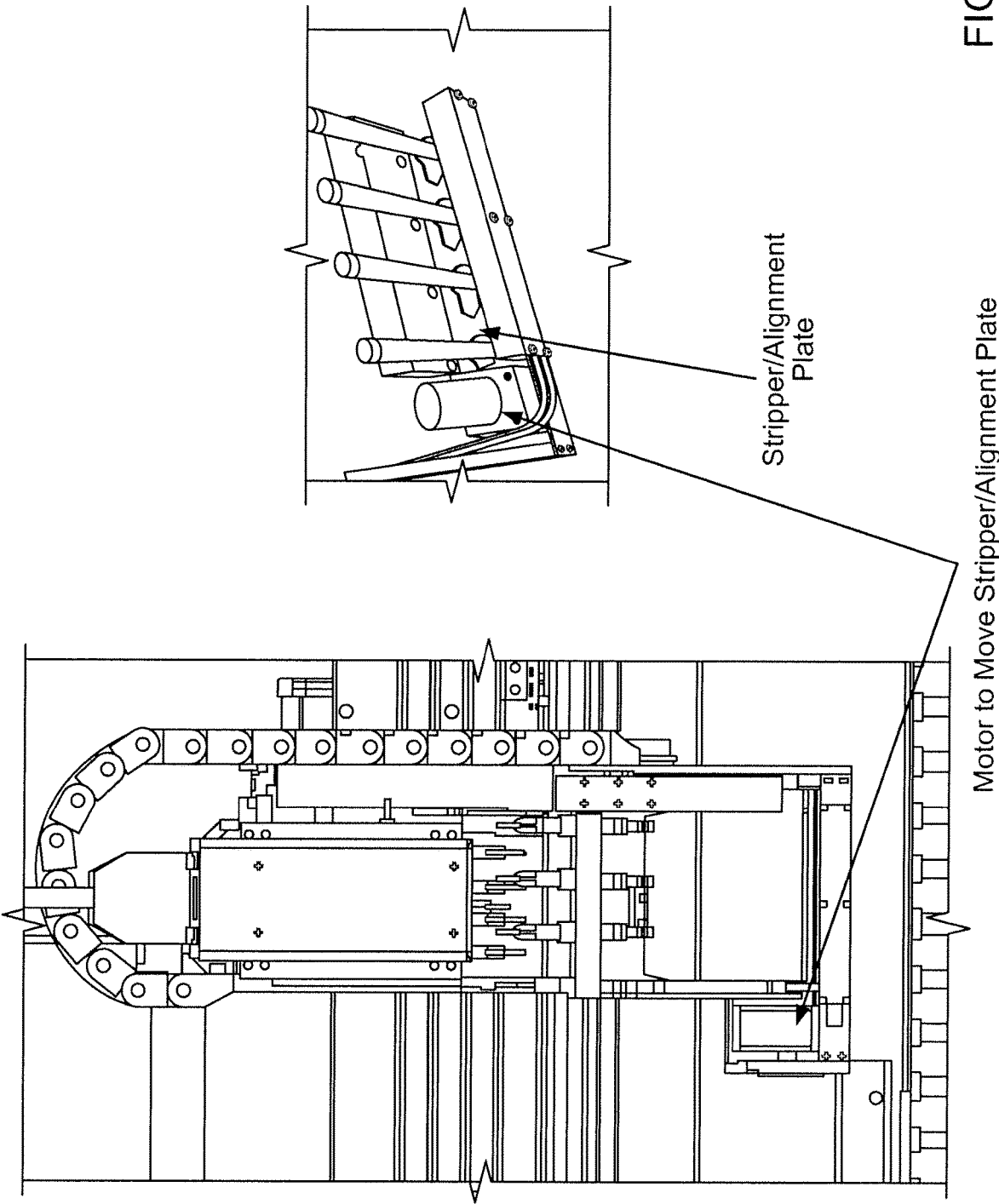


FIG. 25A

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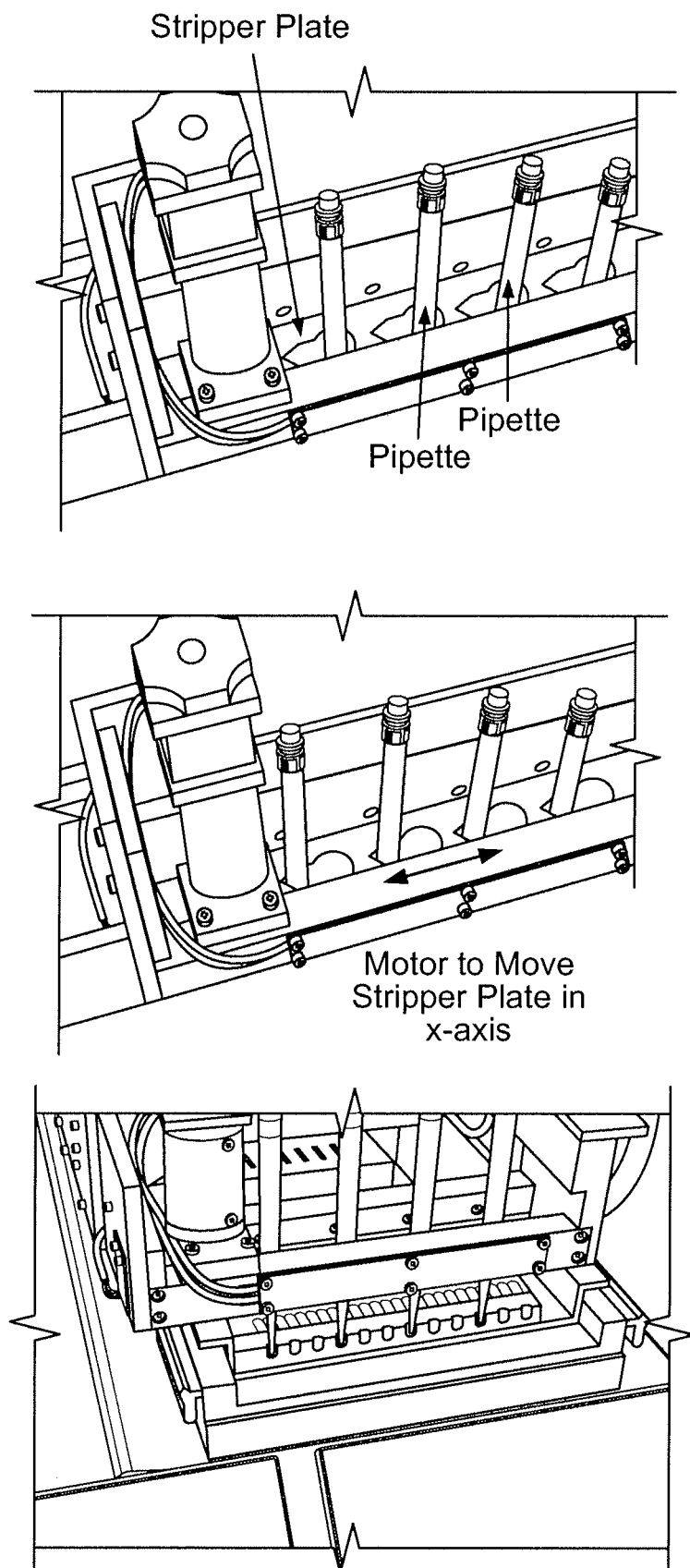


FIG. 25B

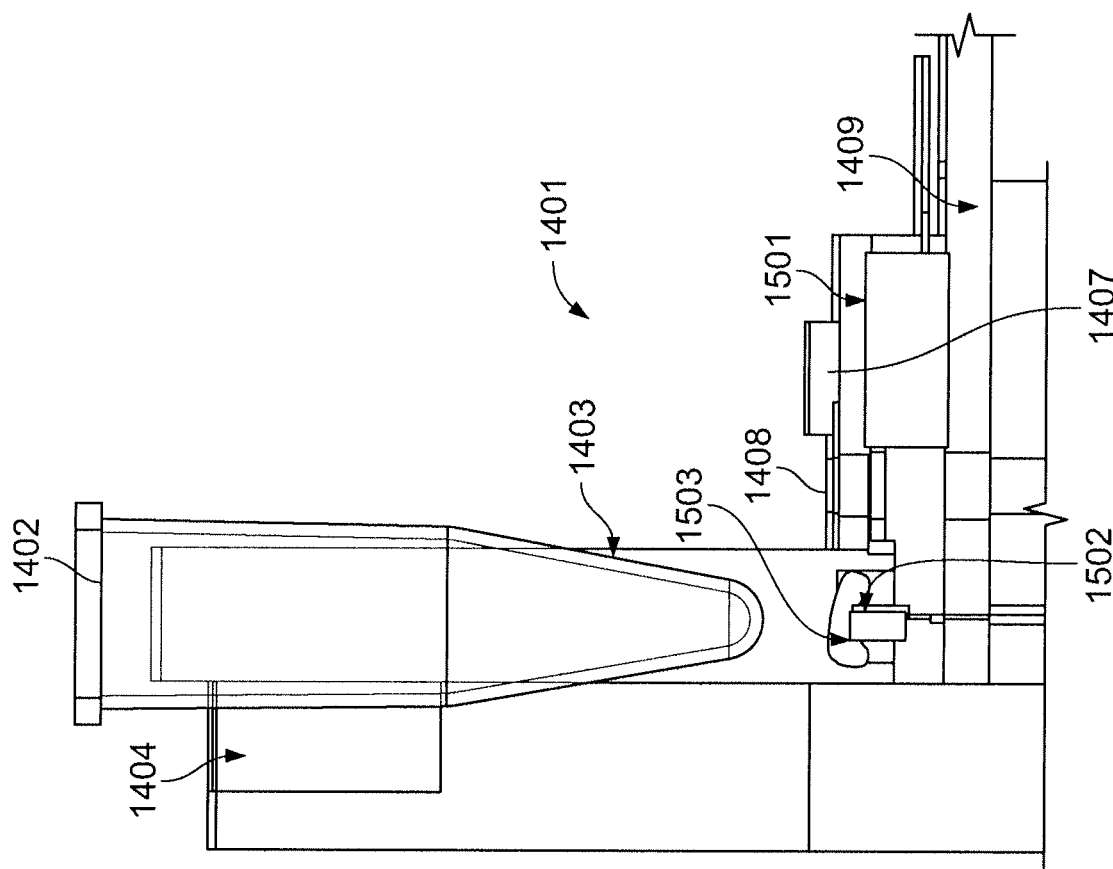
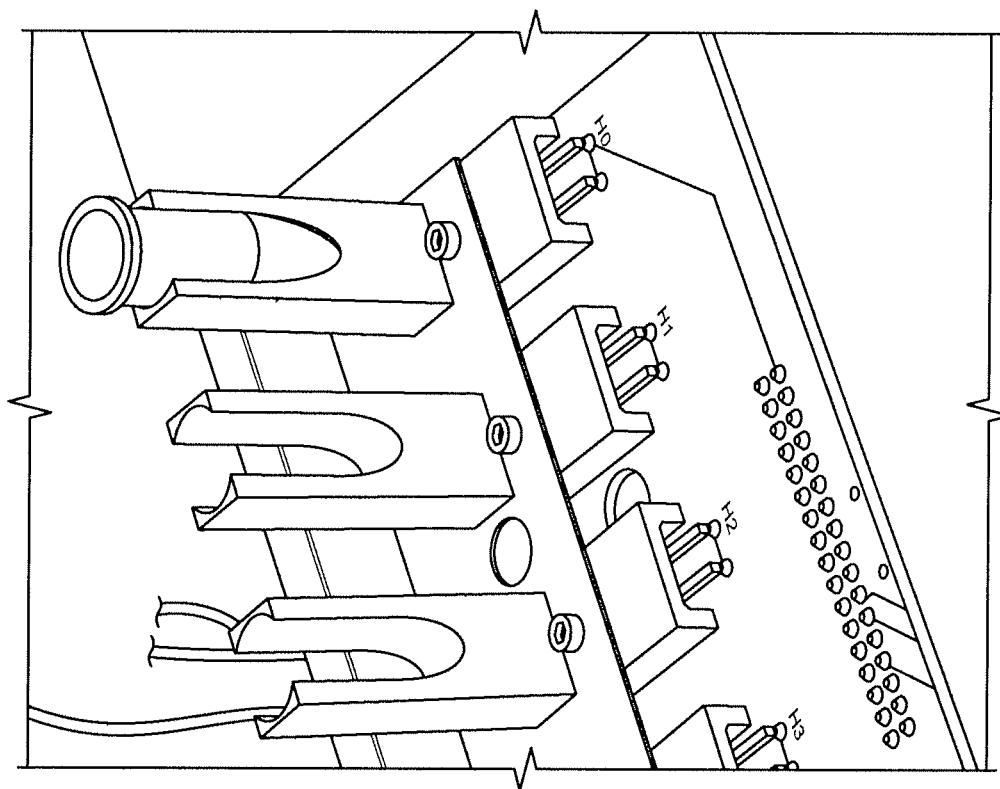


FIG. 26



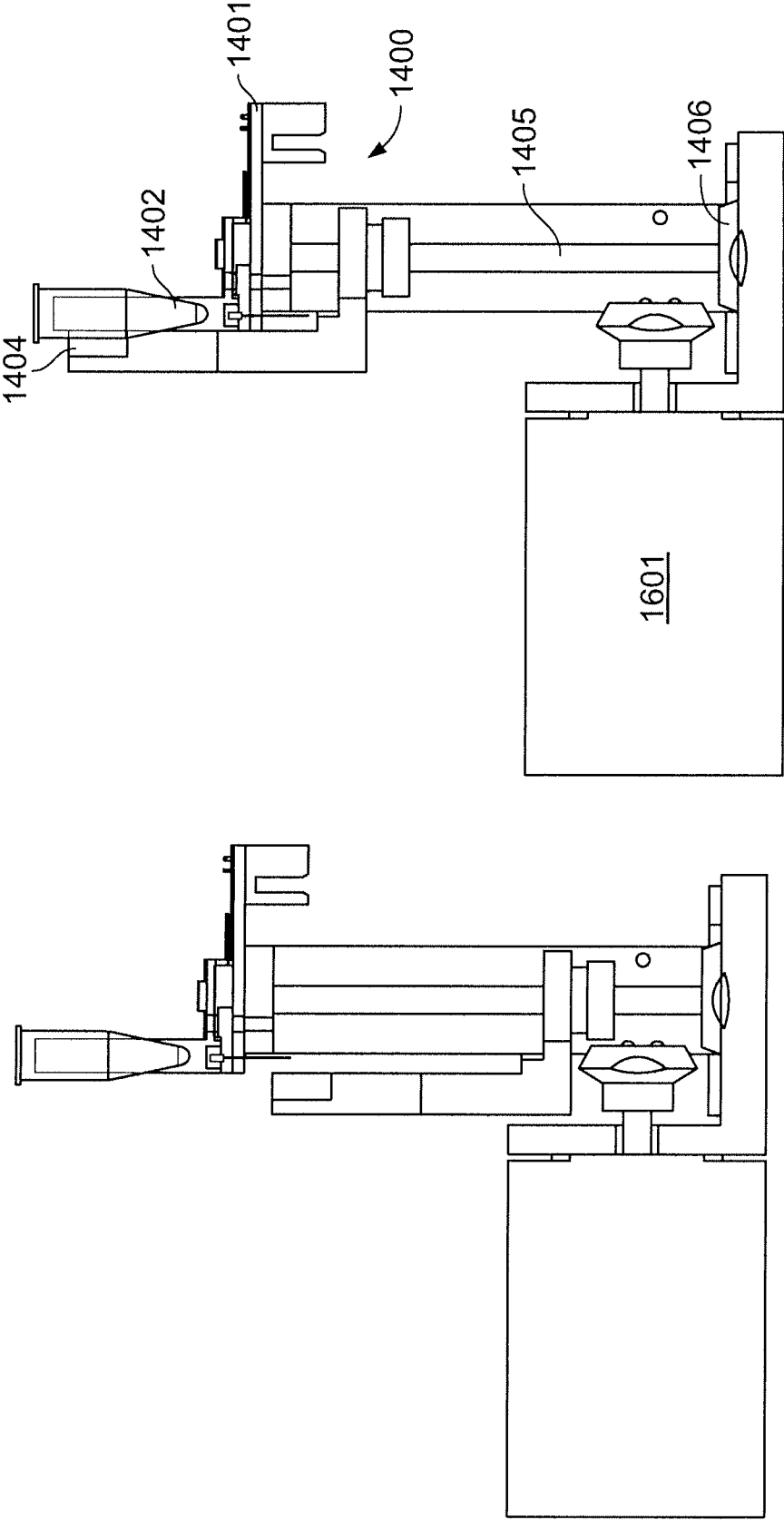


FIG. 27

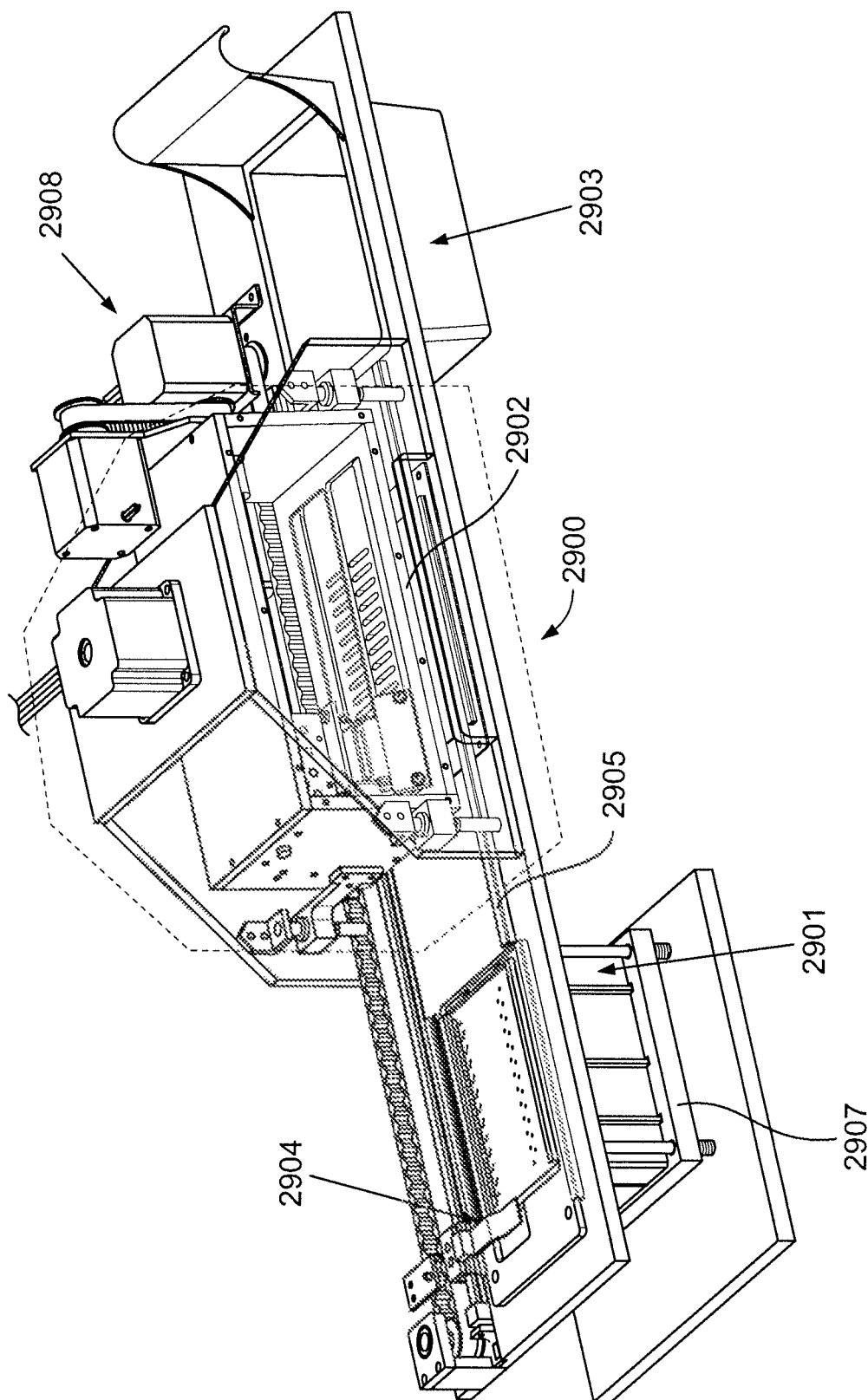


FIG. 28

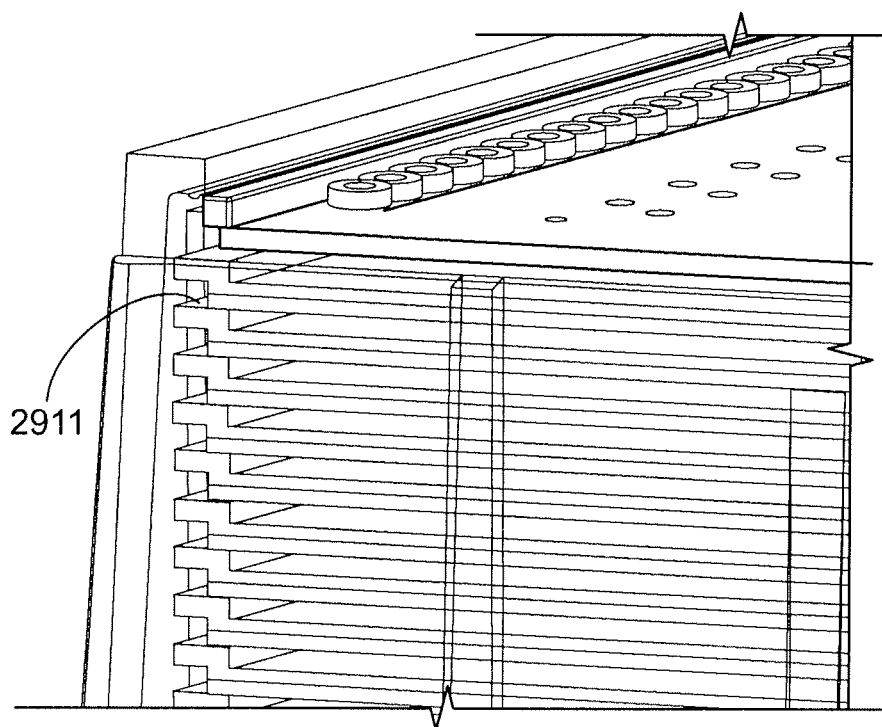
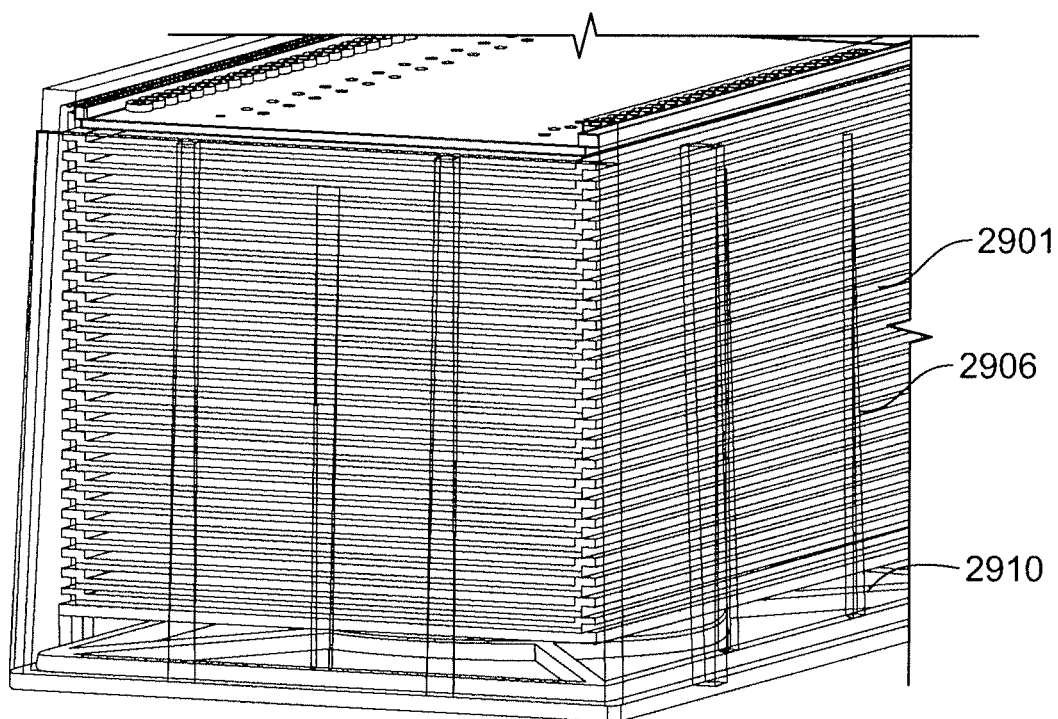


FIG. 29

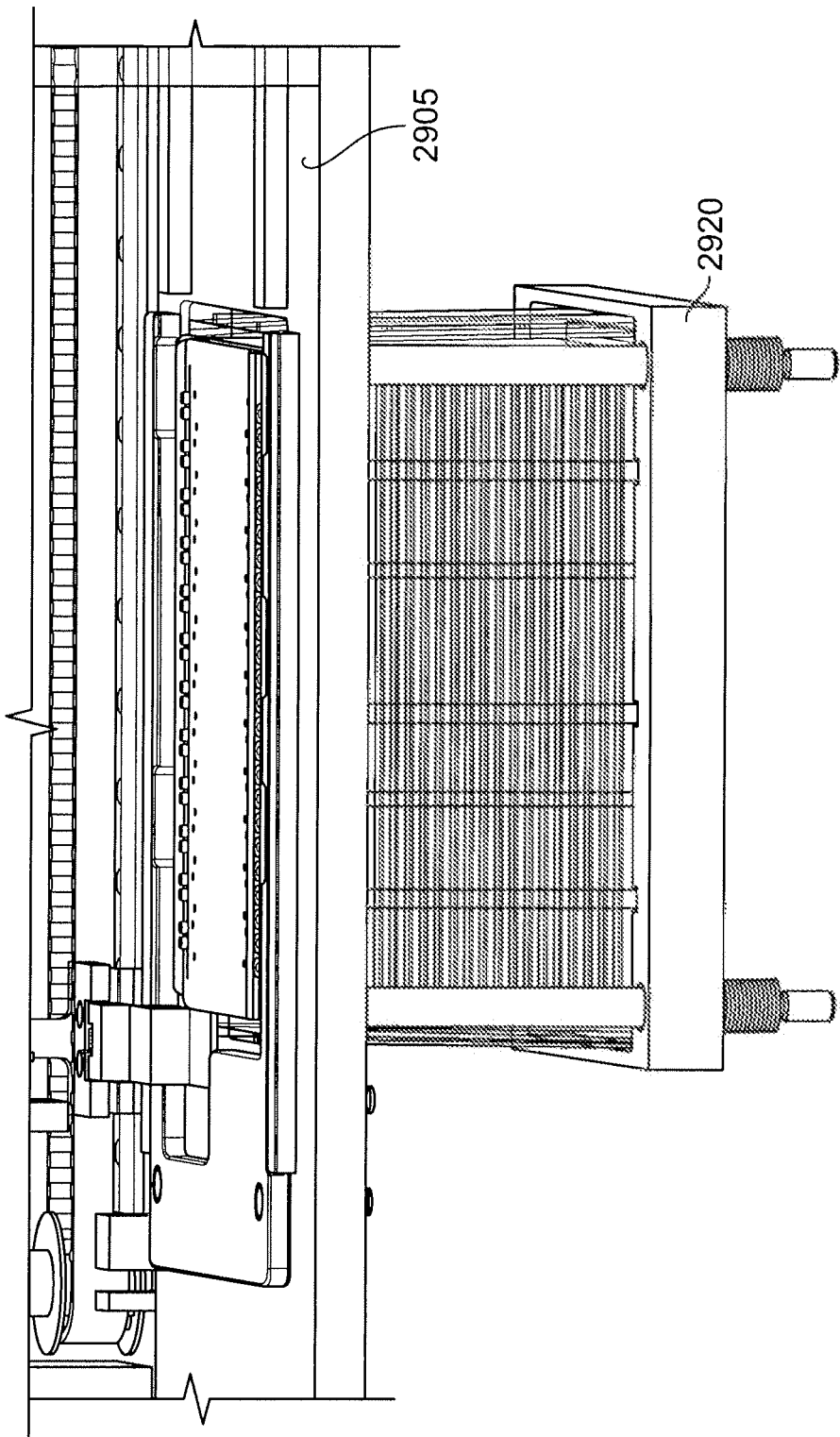


FIG. 30

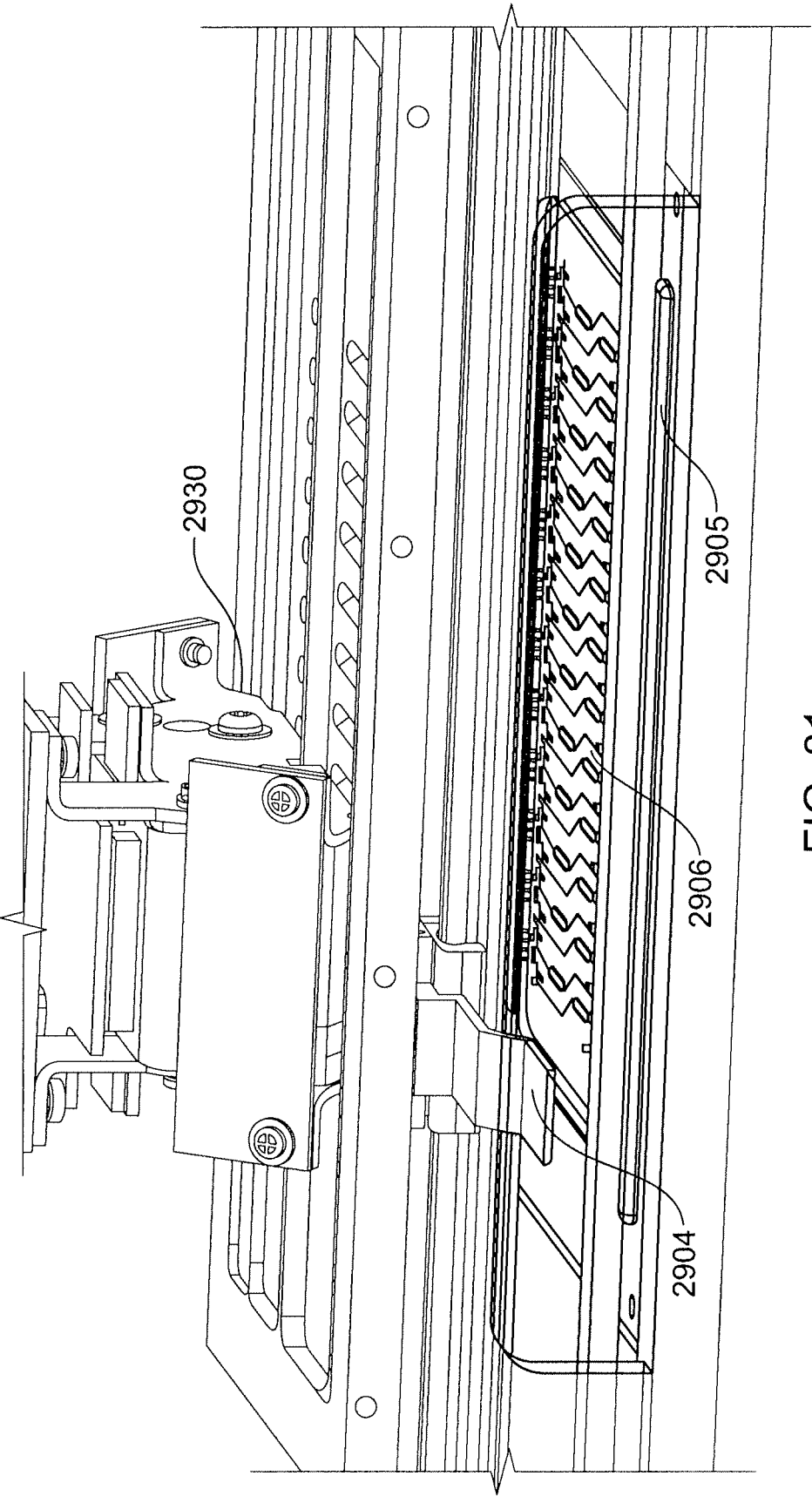


FIG. 31

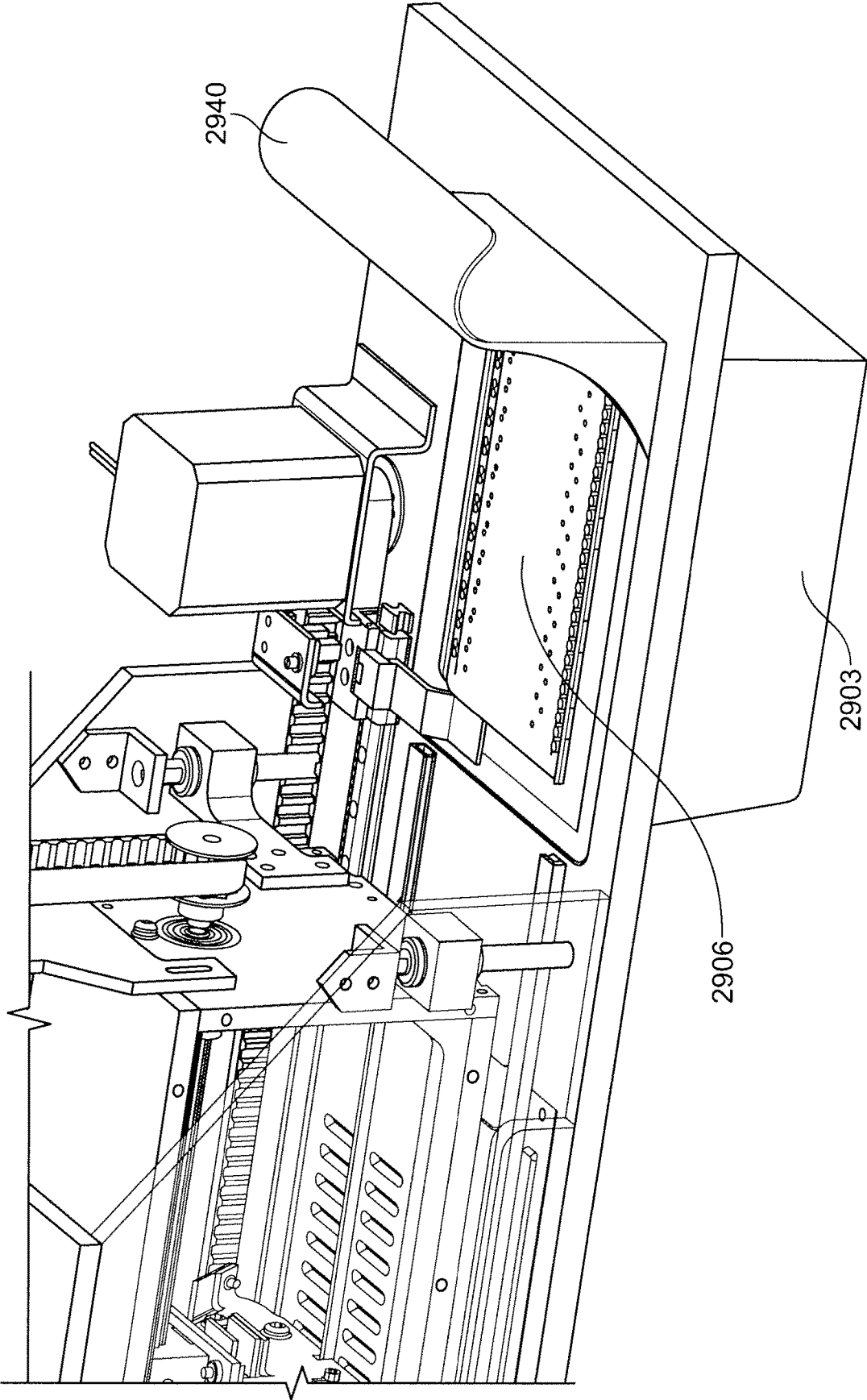


FIG. 32

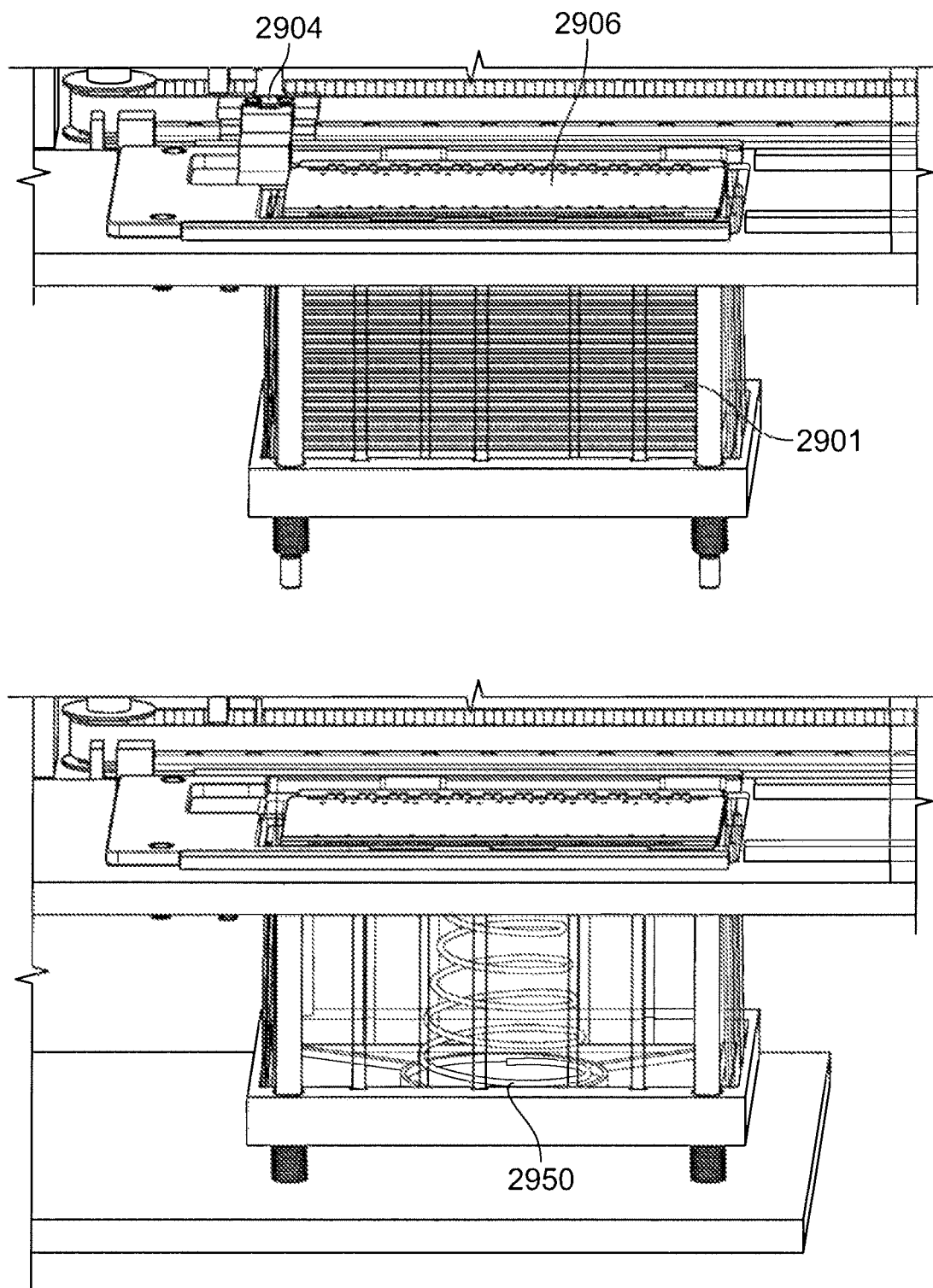
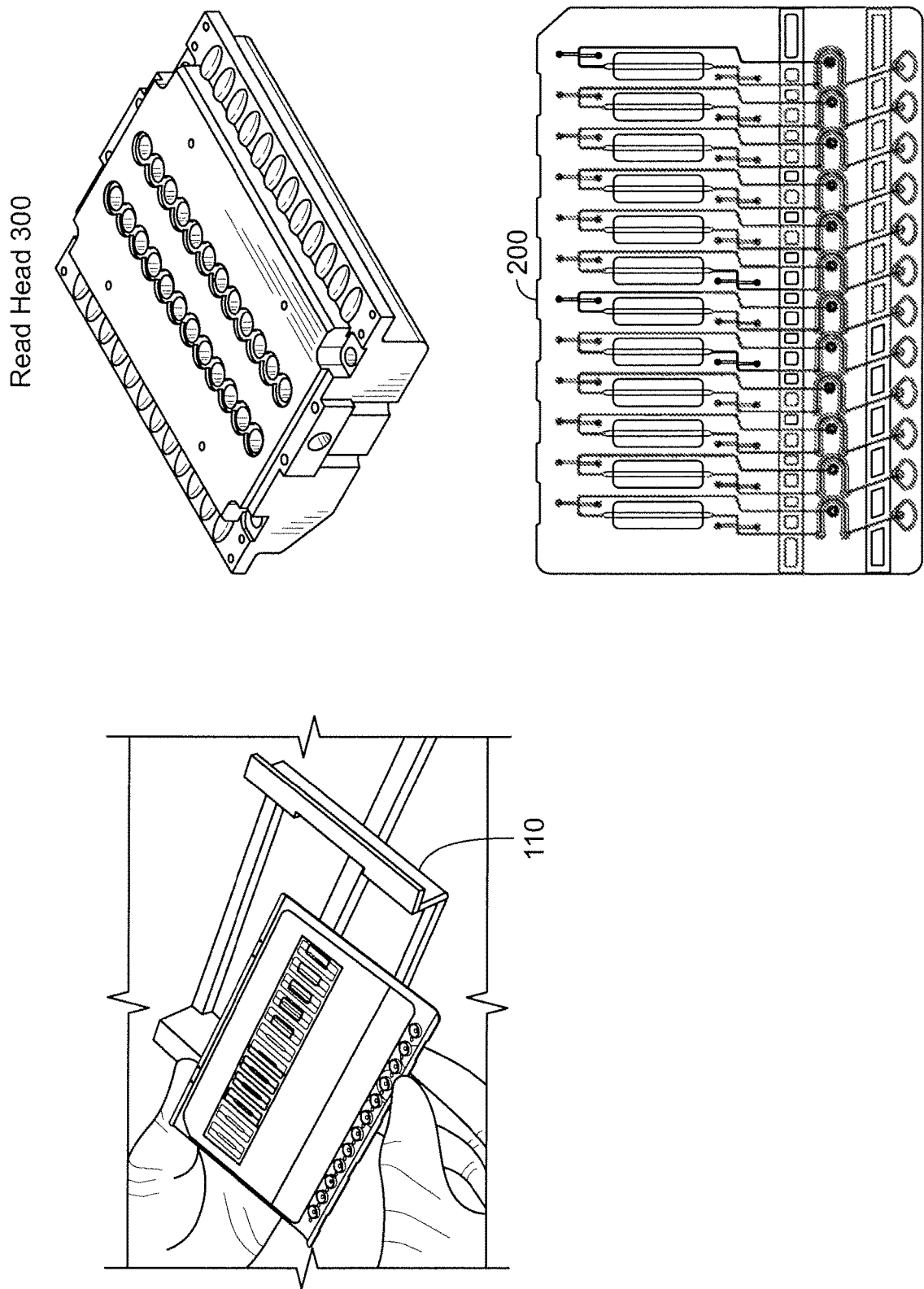


FIG. 33



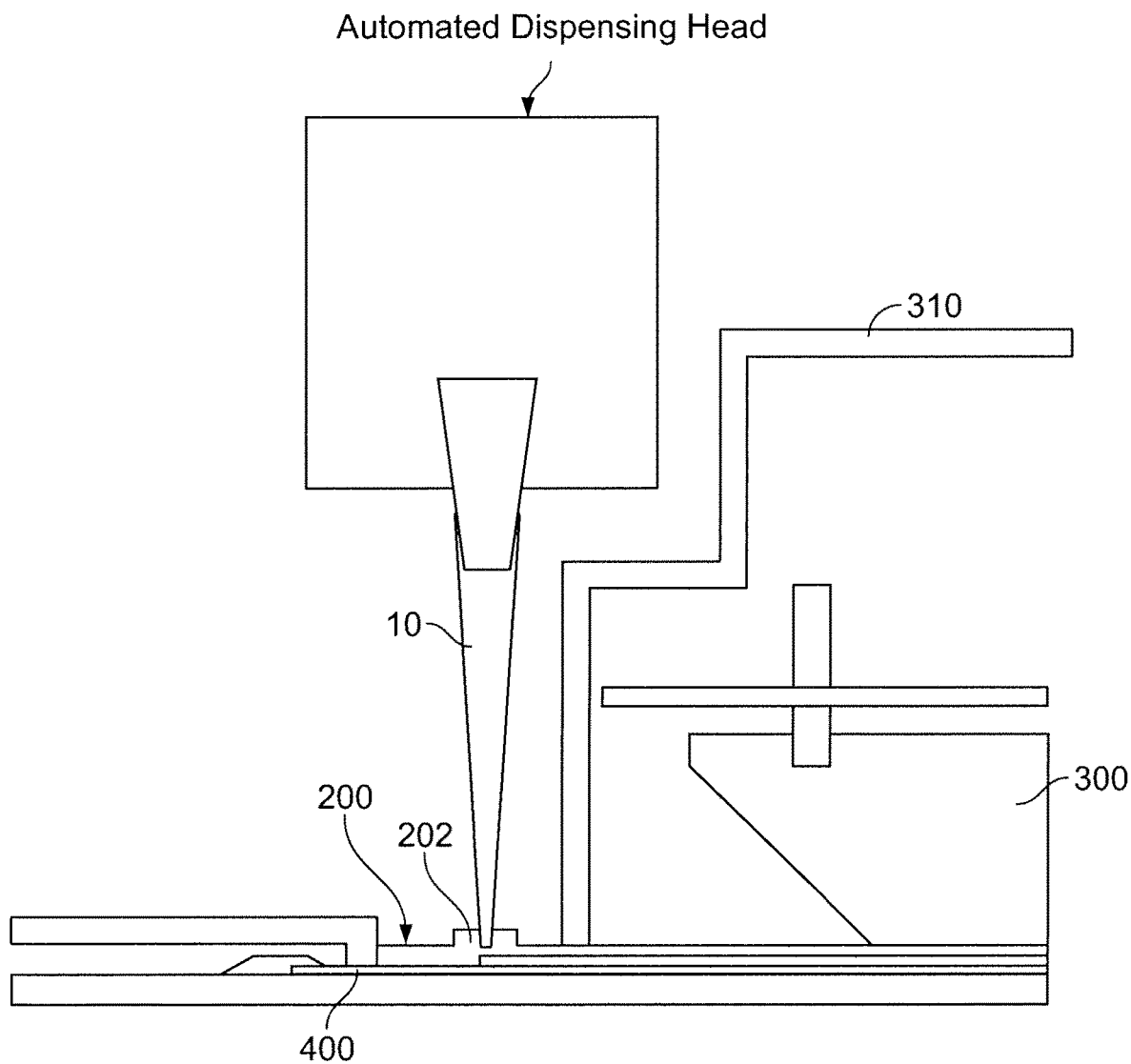


FIG. 35

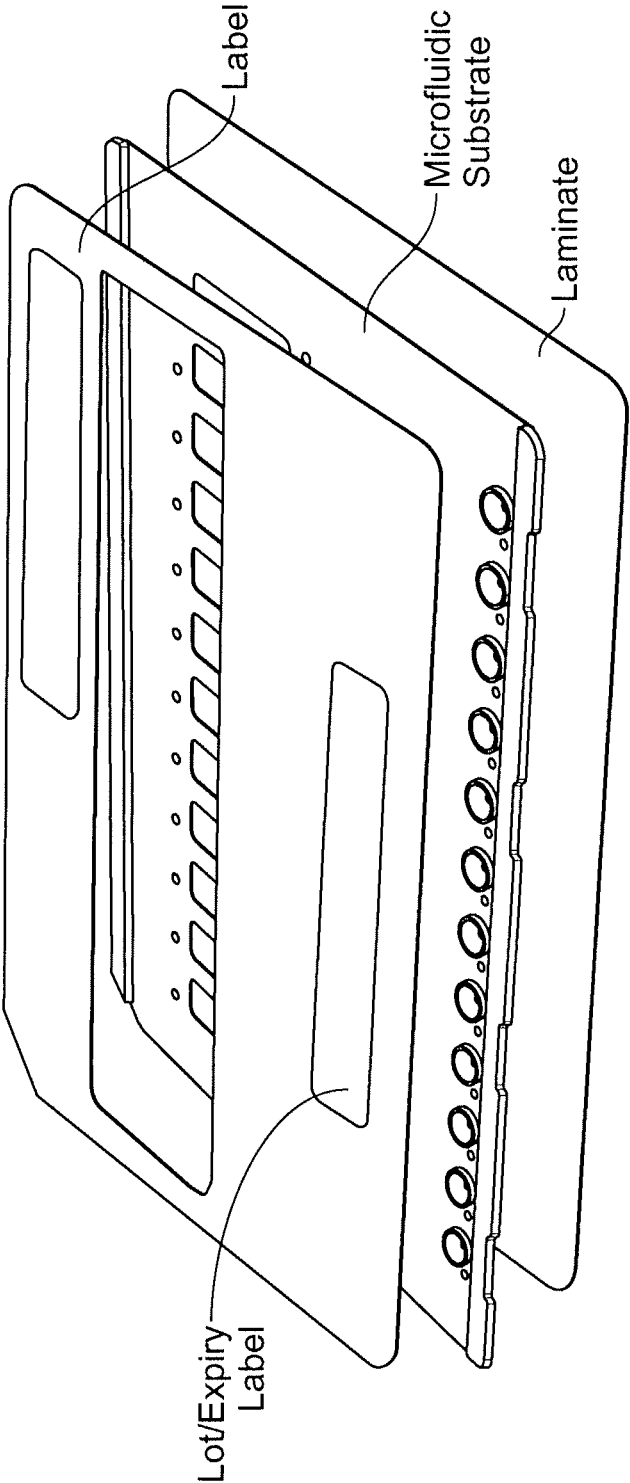


FIG. 36

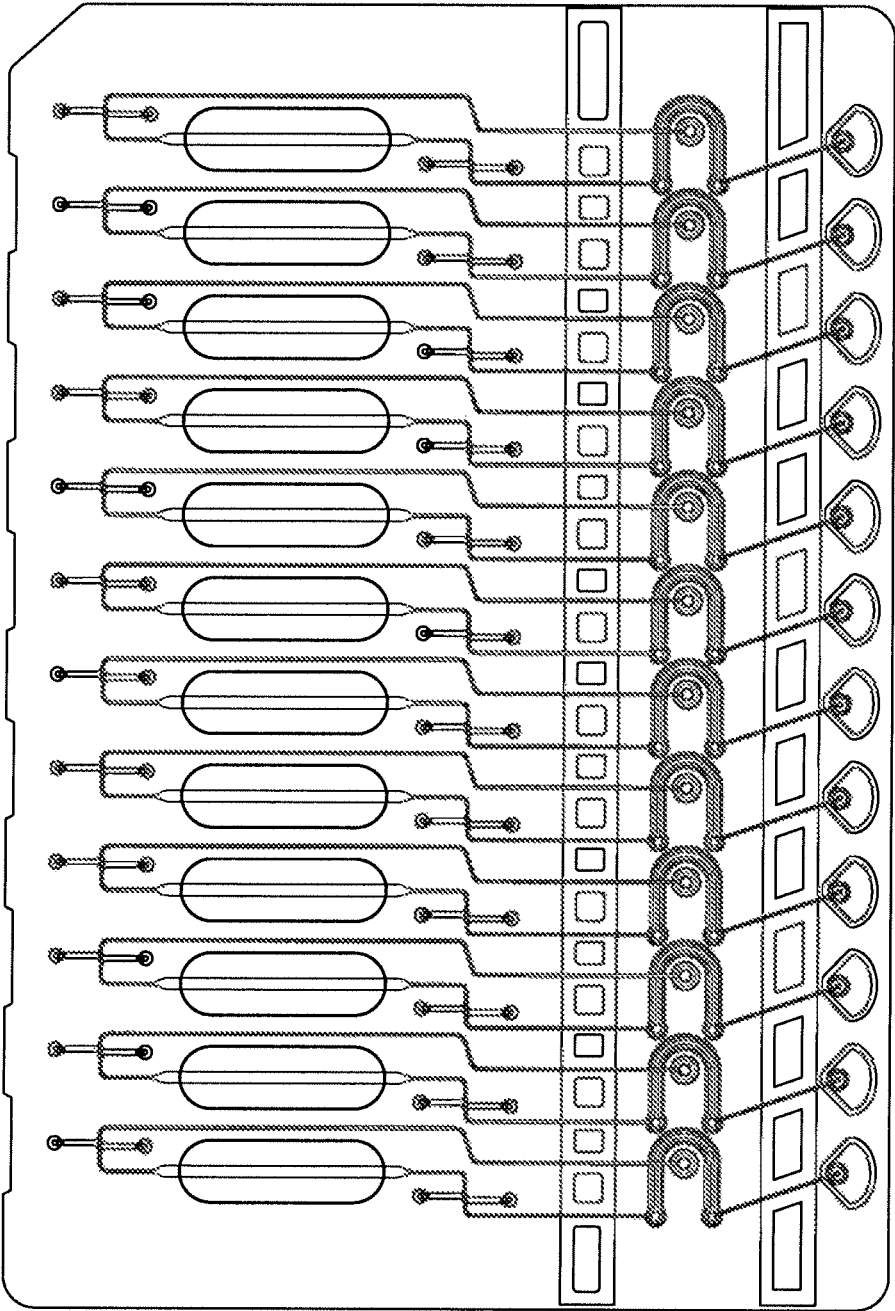


FIG. 37

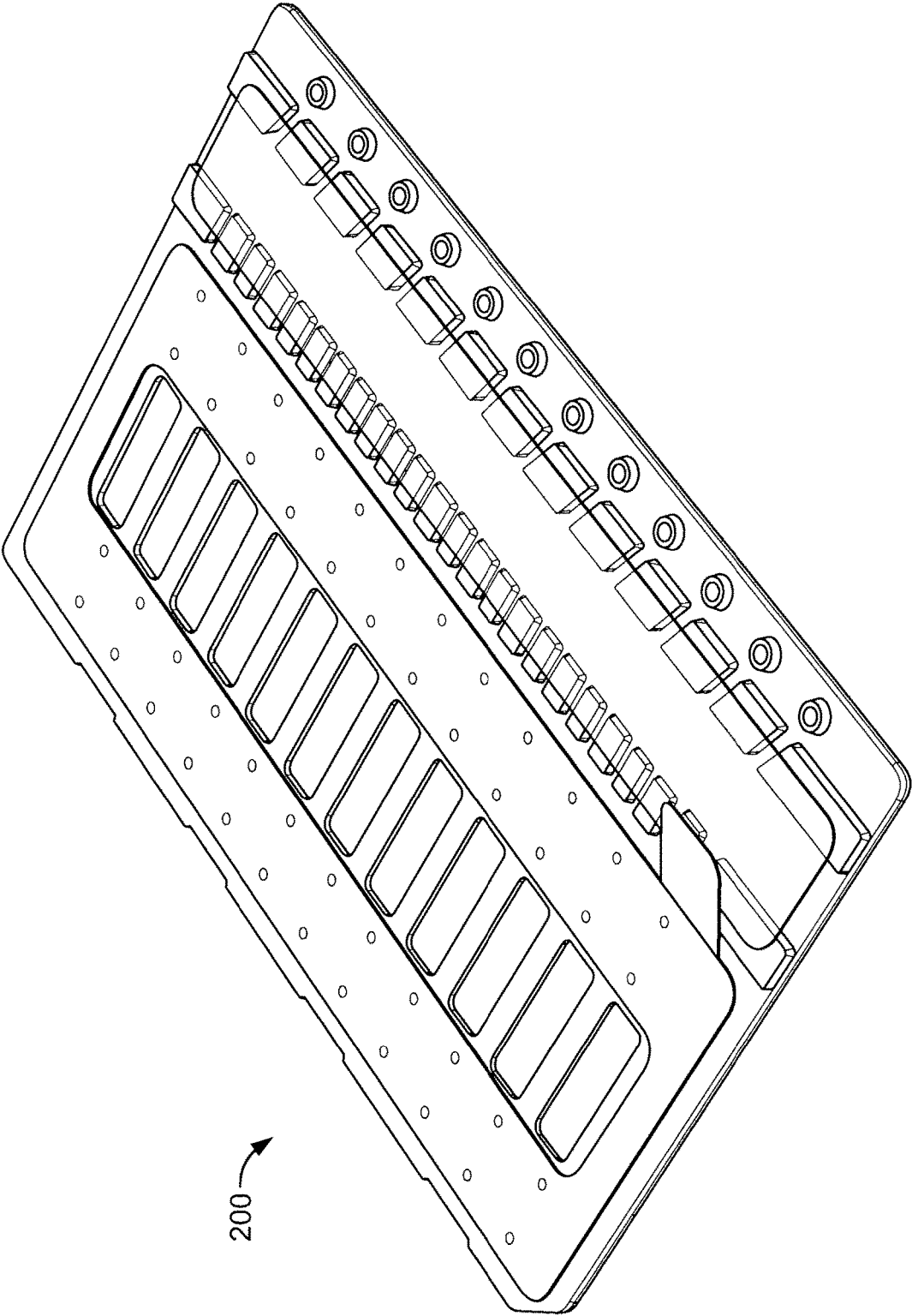


FIG. 38A

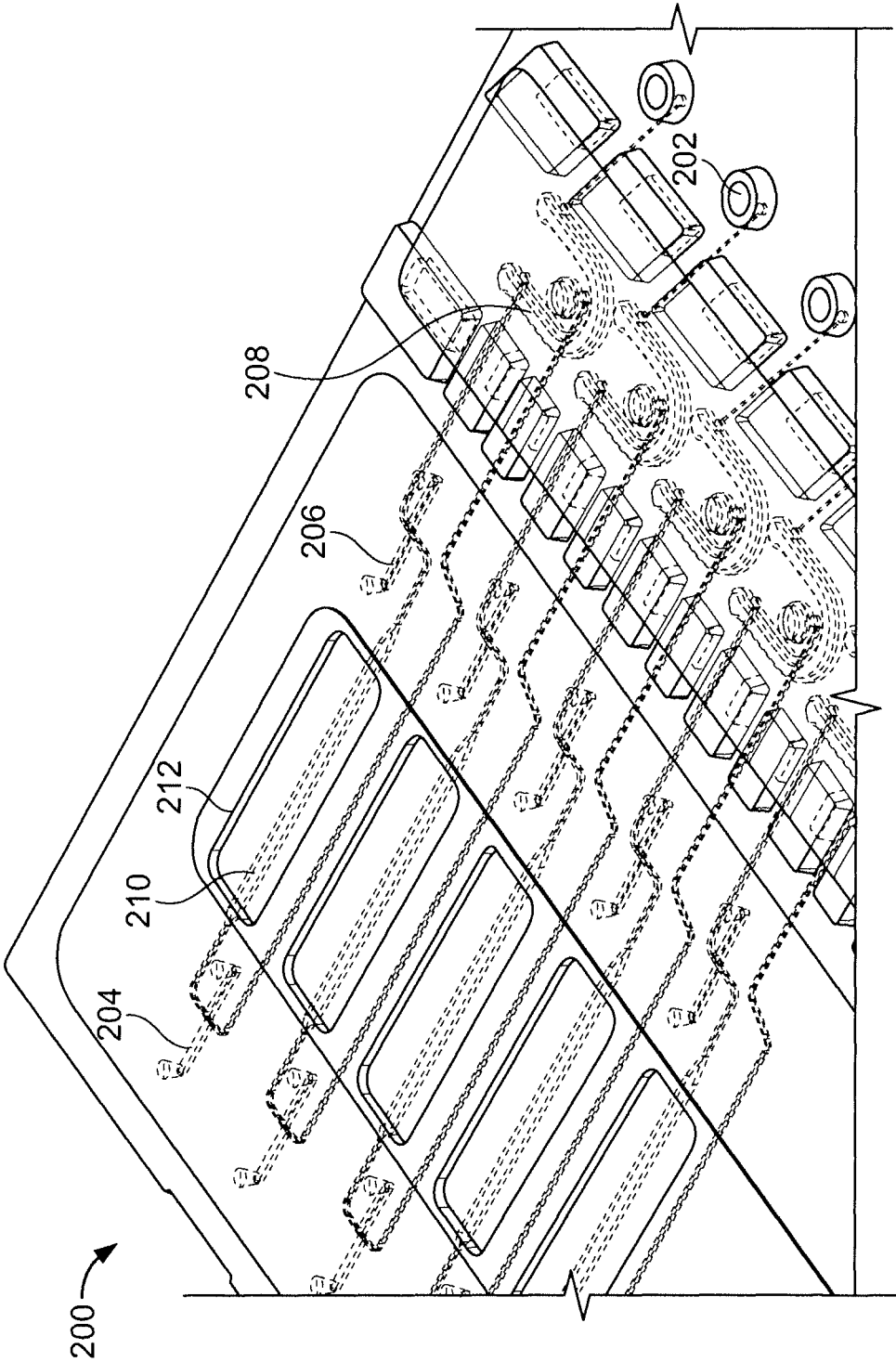


FIG. 38B

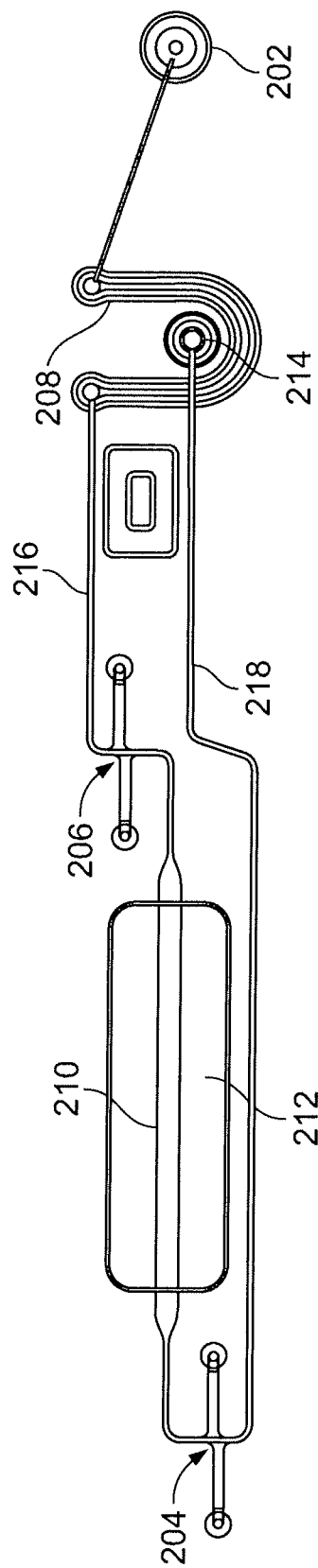


FIG. 39A

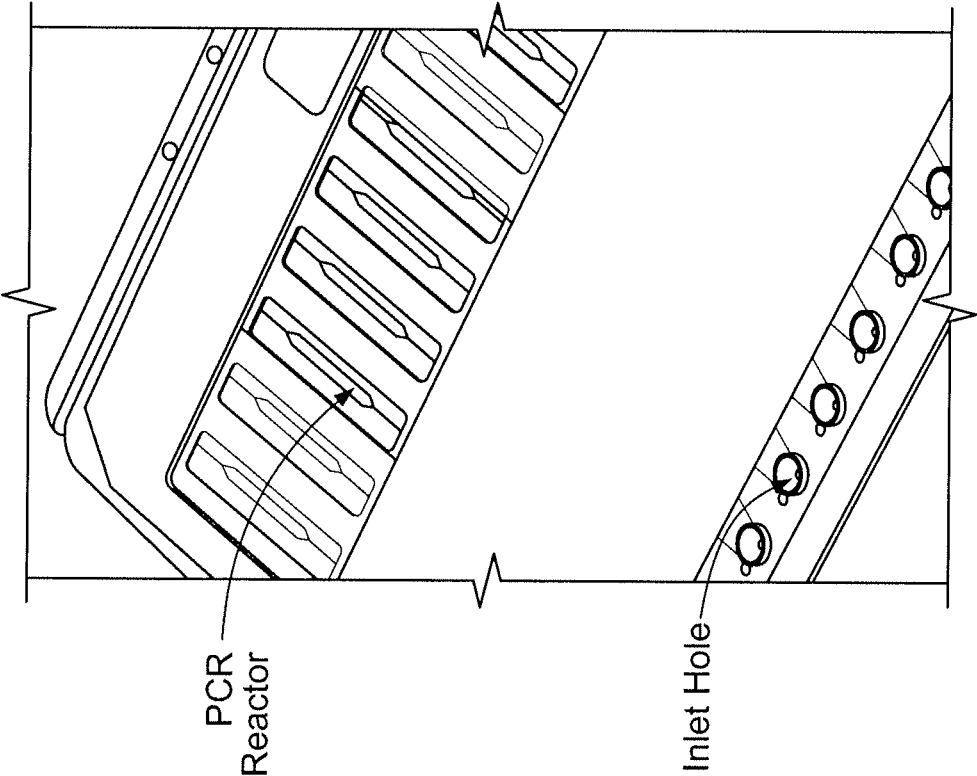
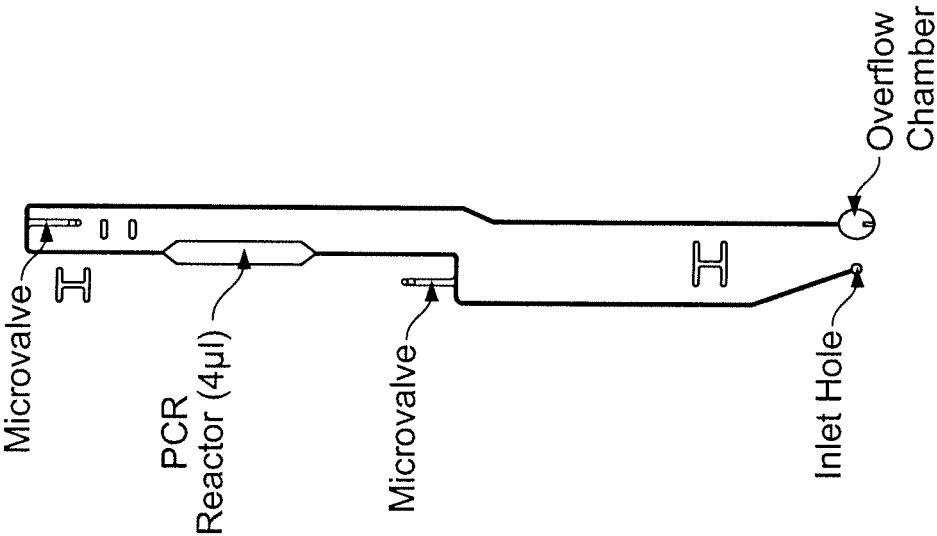


FIG. 39B



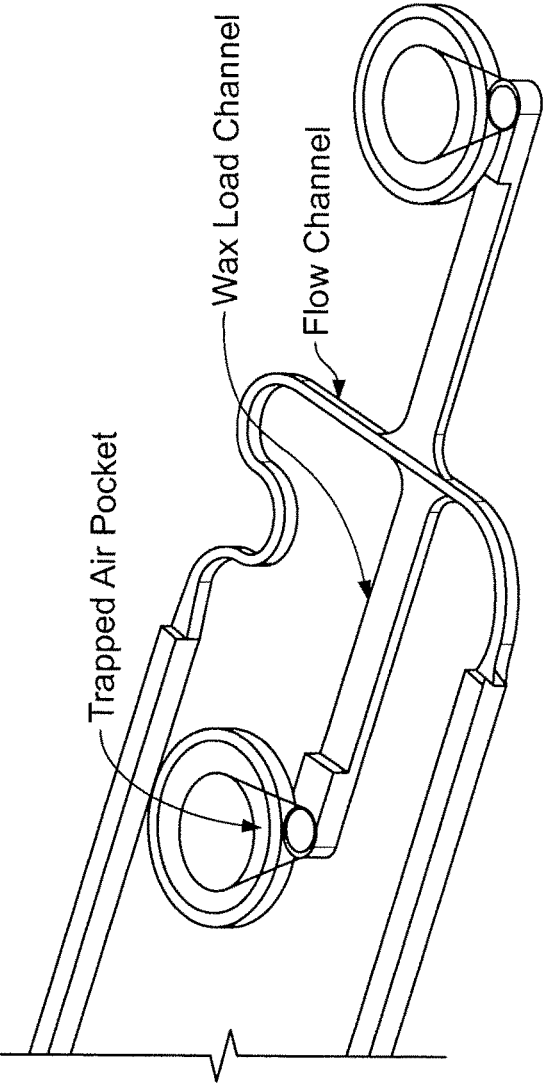
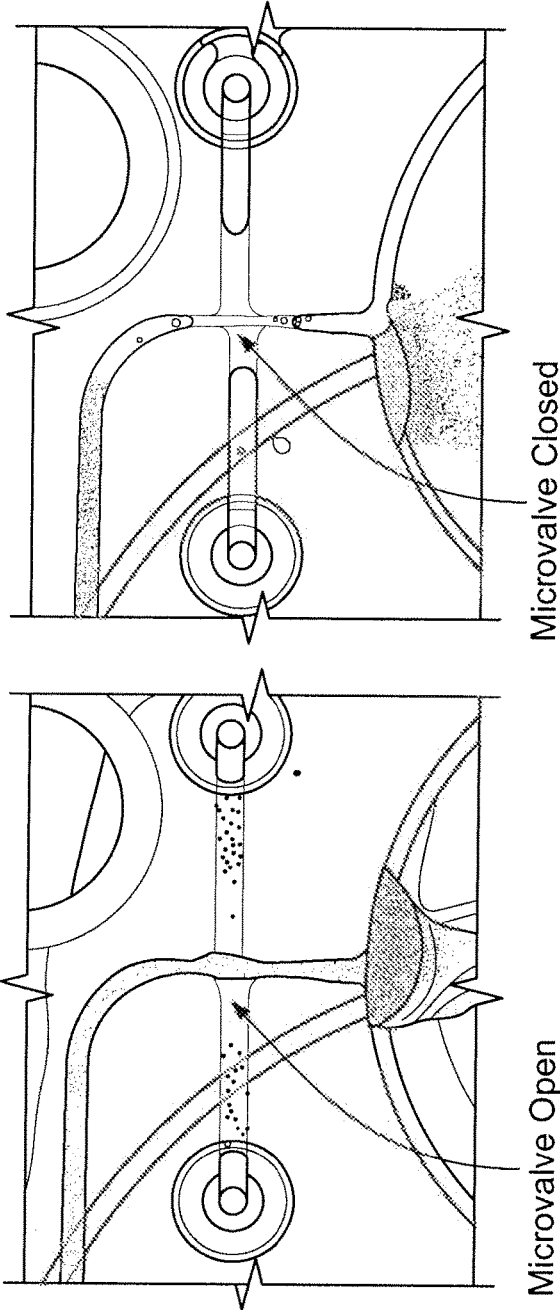


FIG. 40A

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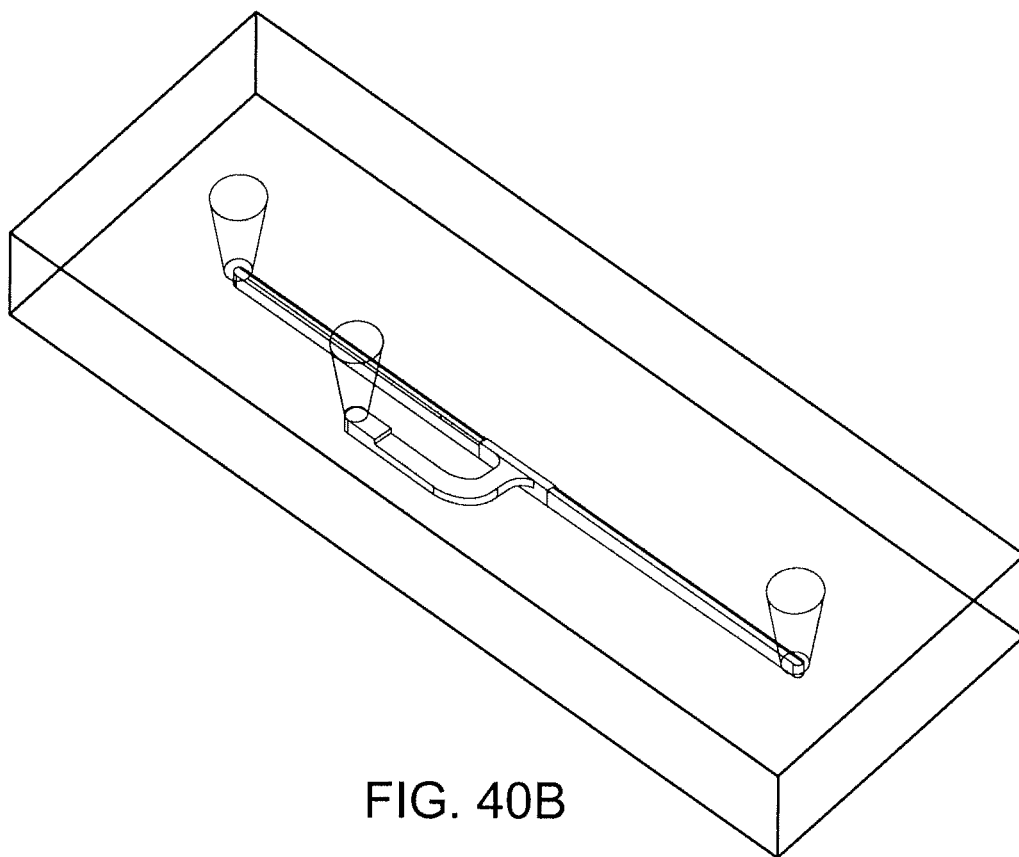


FIG. 40B

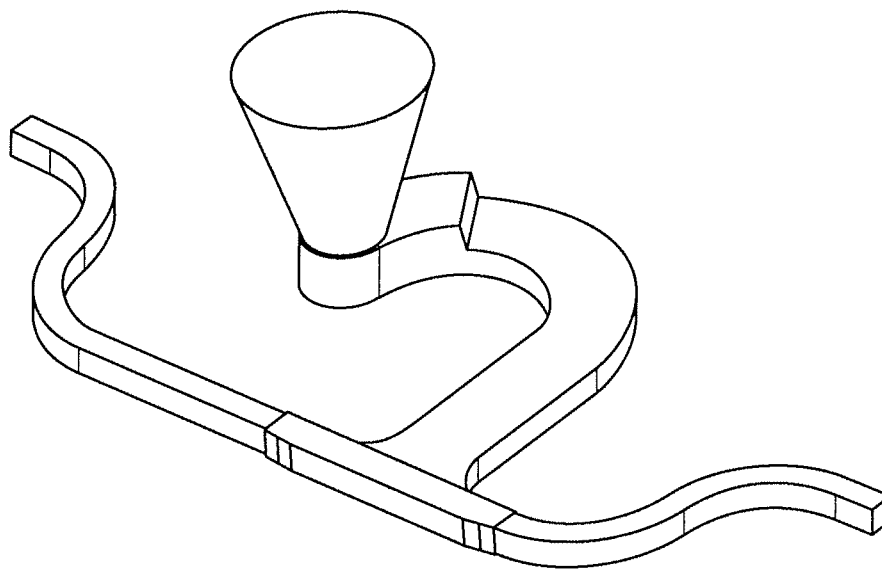


FIG. 40C

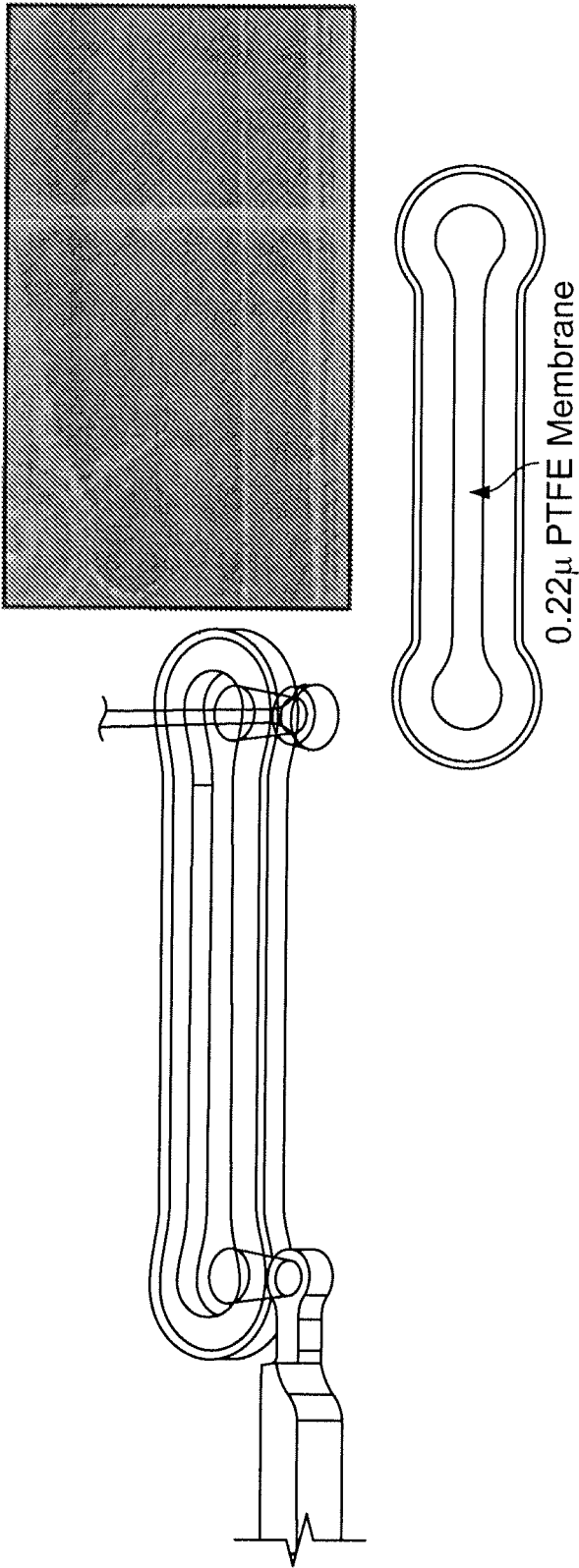


FIG. 41

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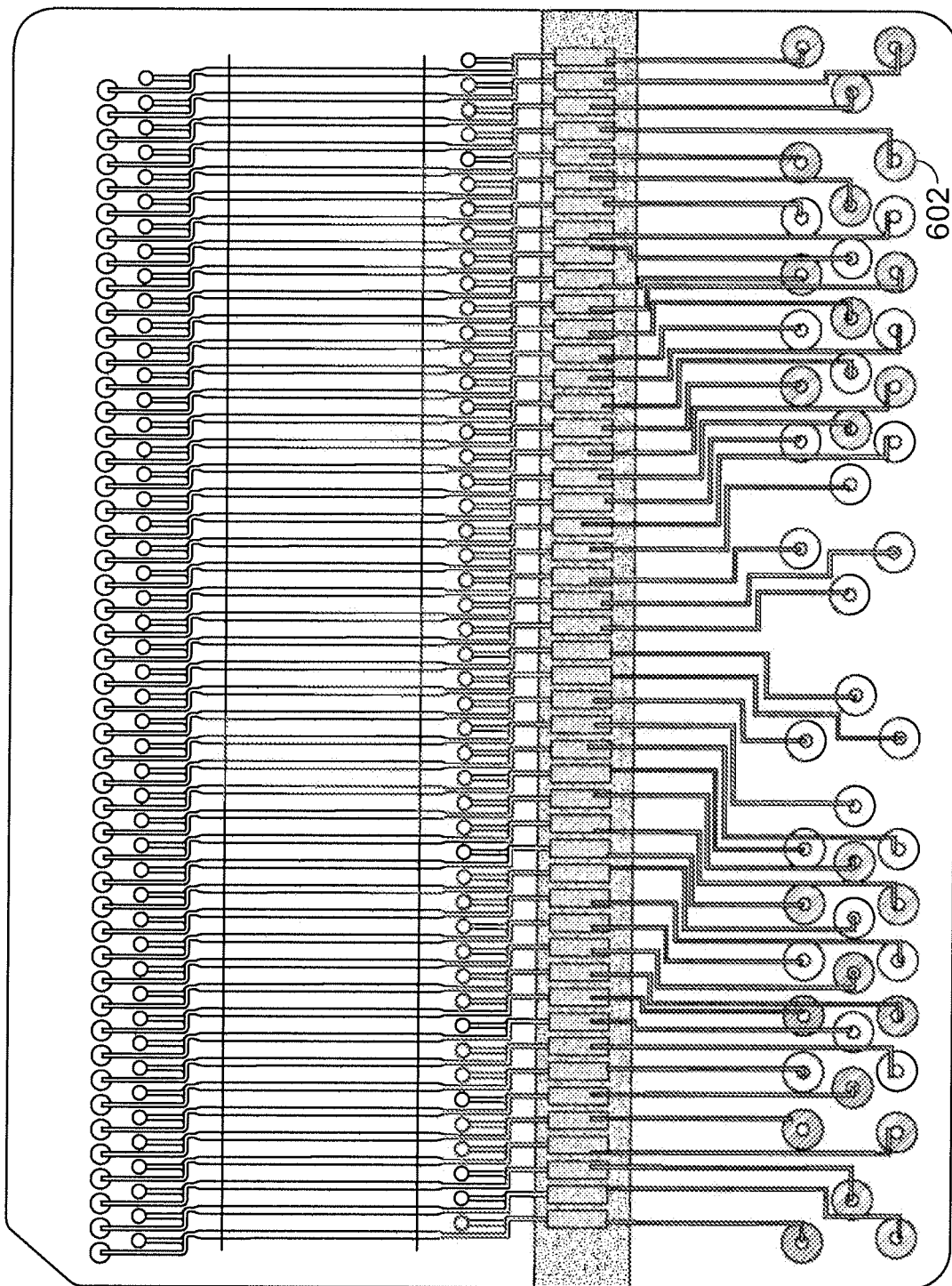


FIG. 42

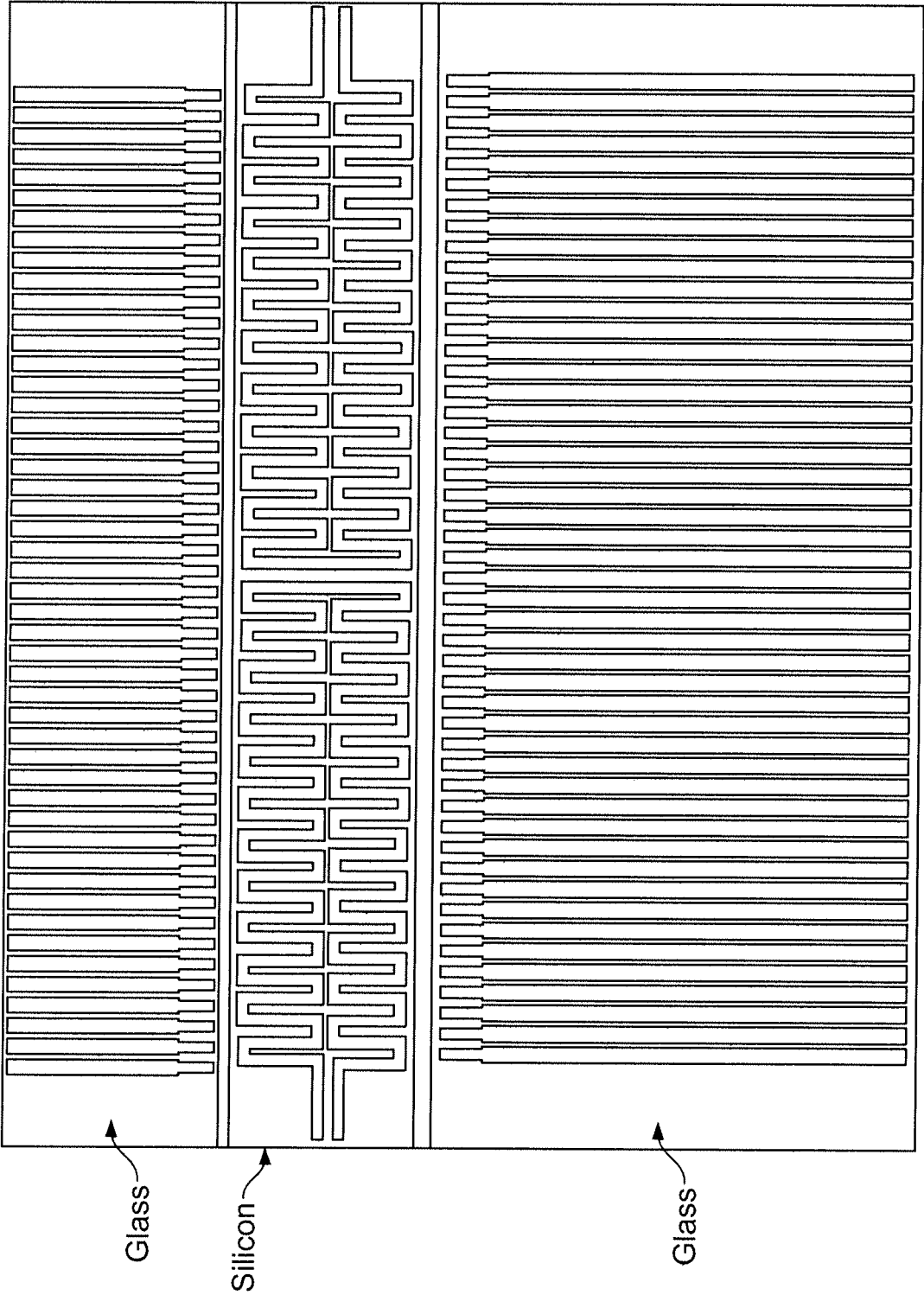


FIG. 43

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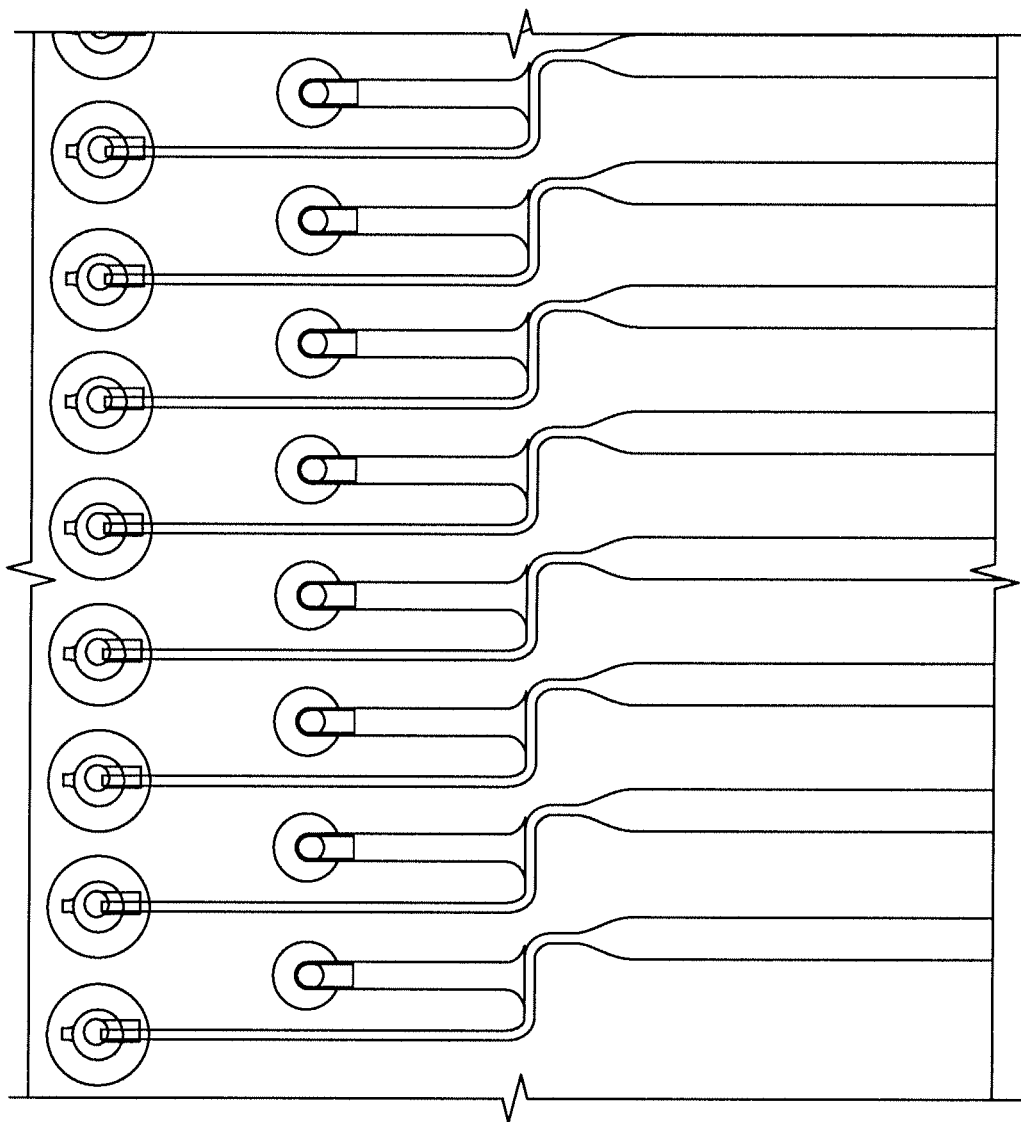


FIG. 44

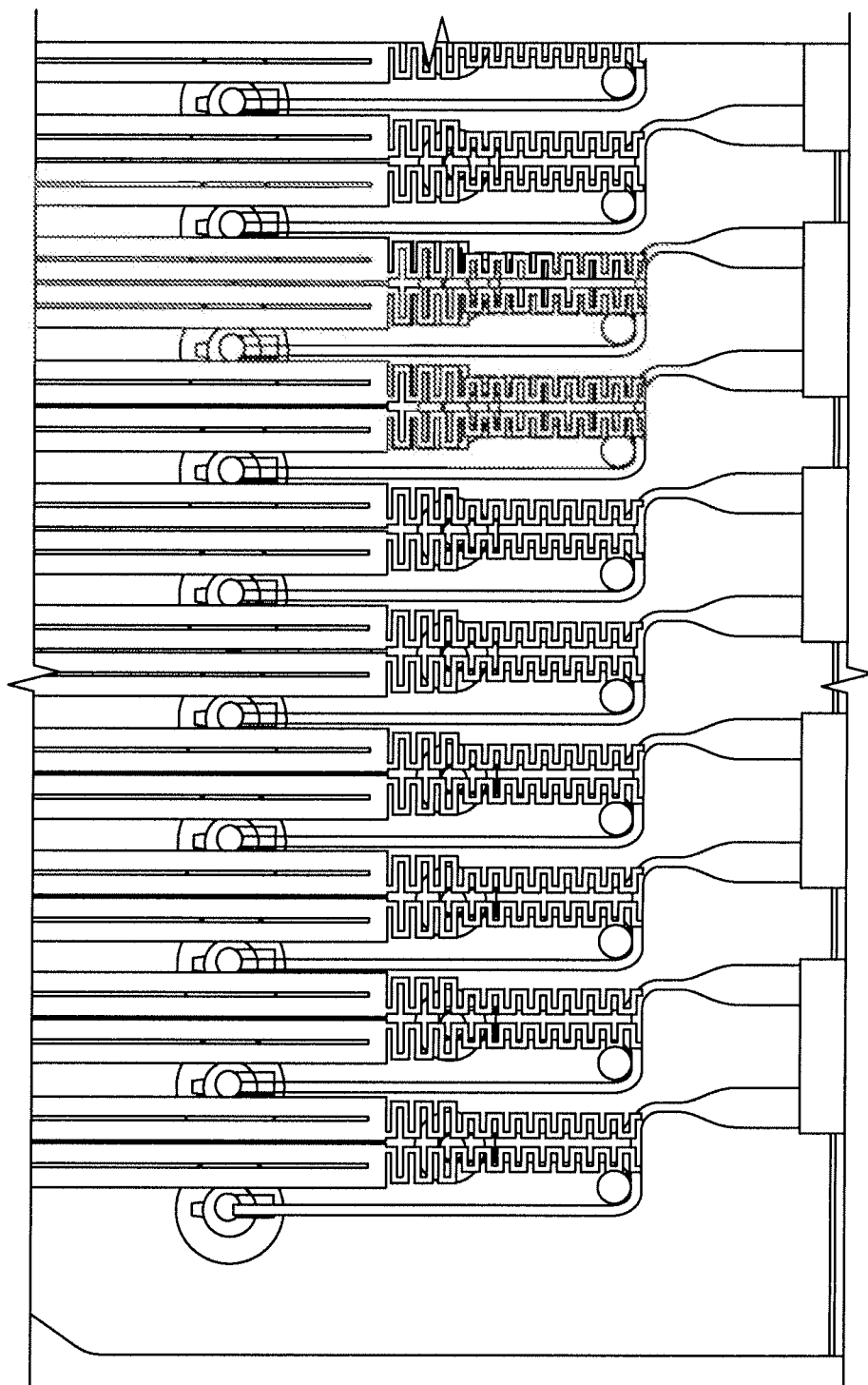


FIG. 45

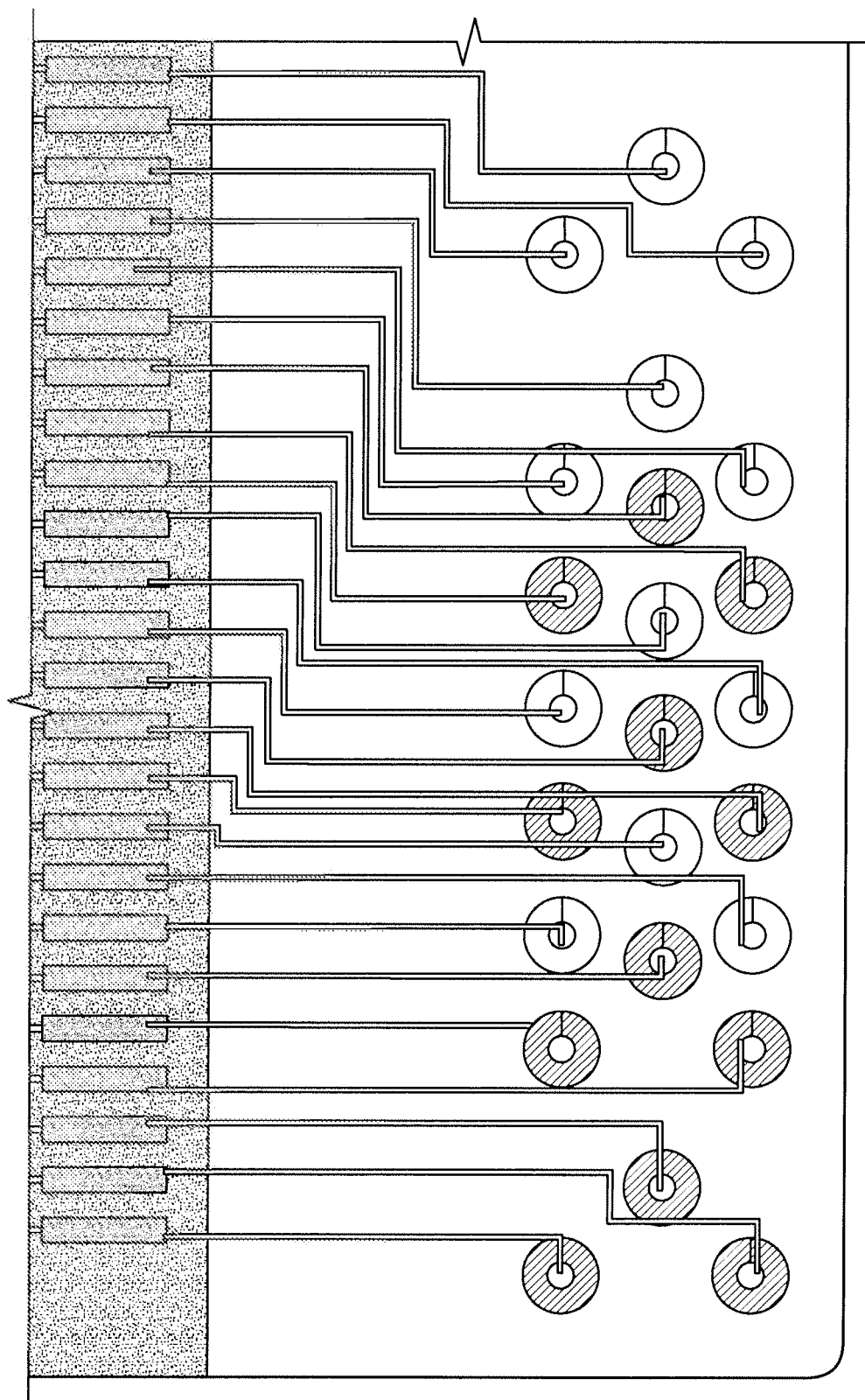


FIG. 46

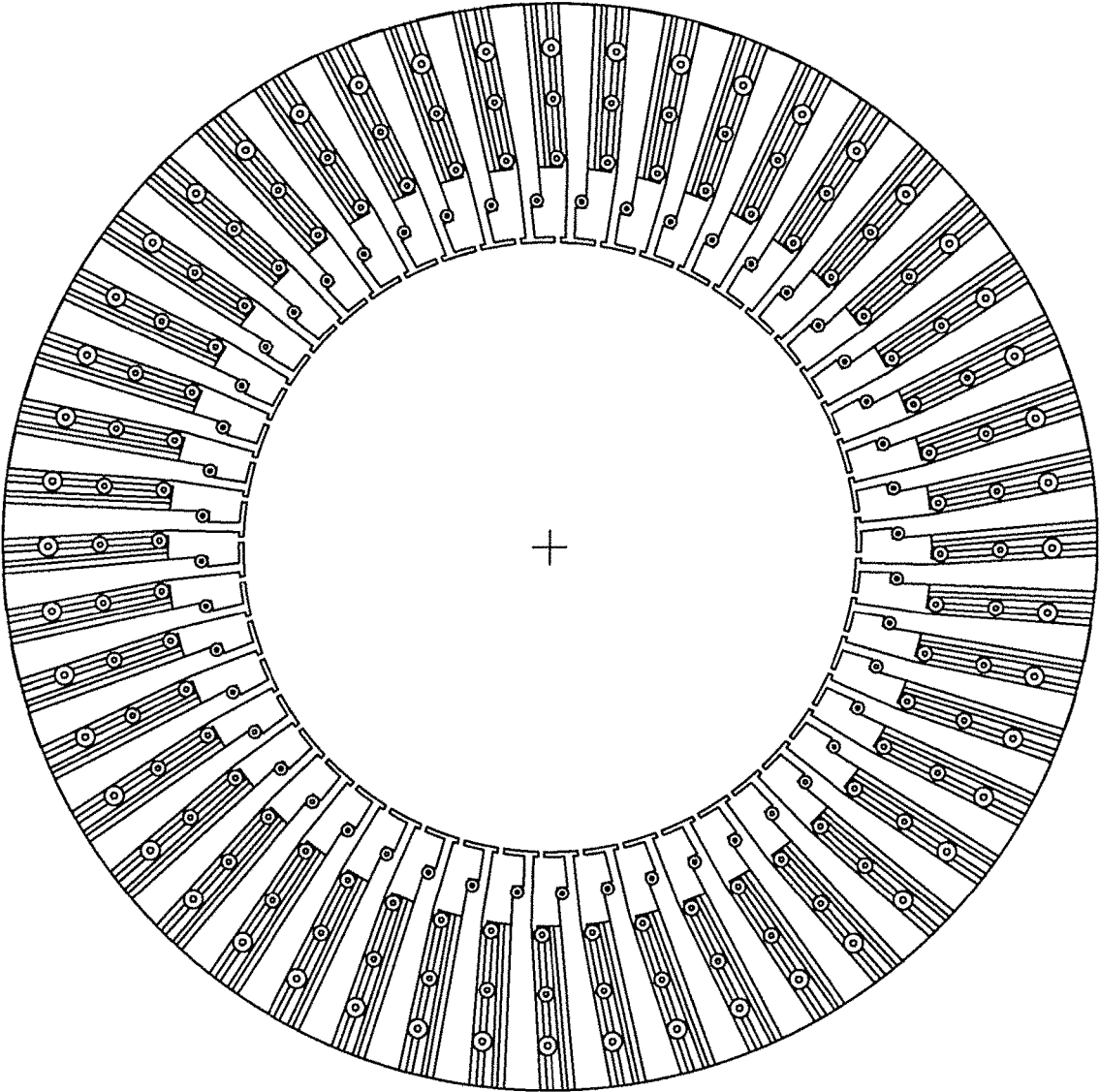


FIG. 47A

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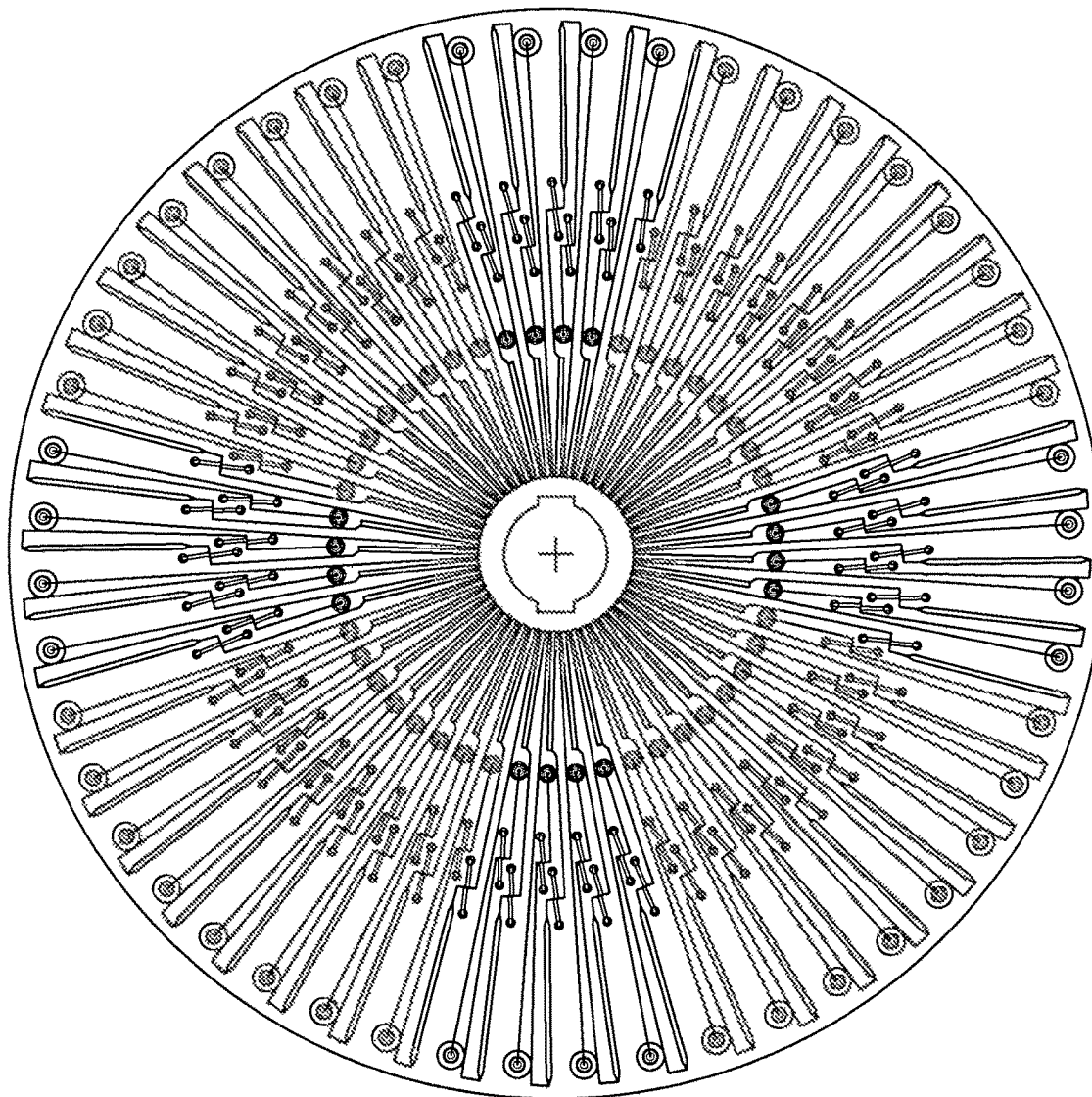


FIG. 47B

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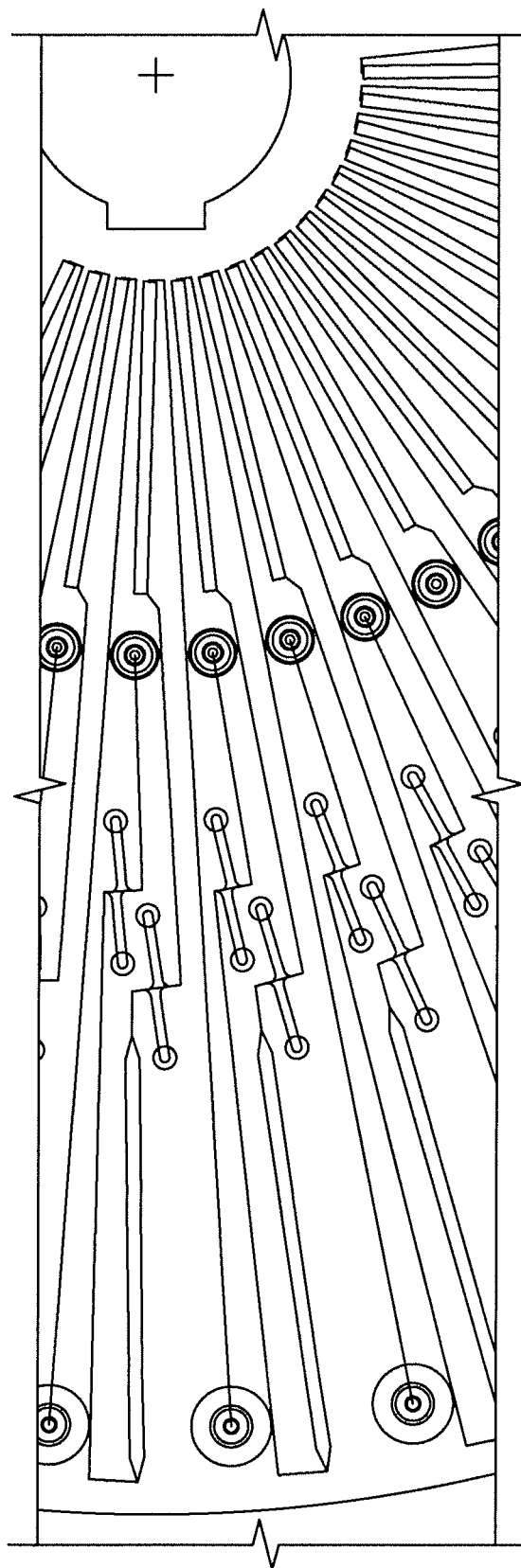


FIG. 47C

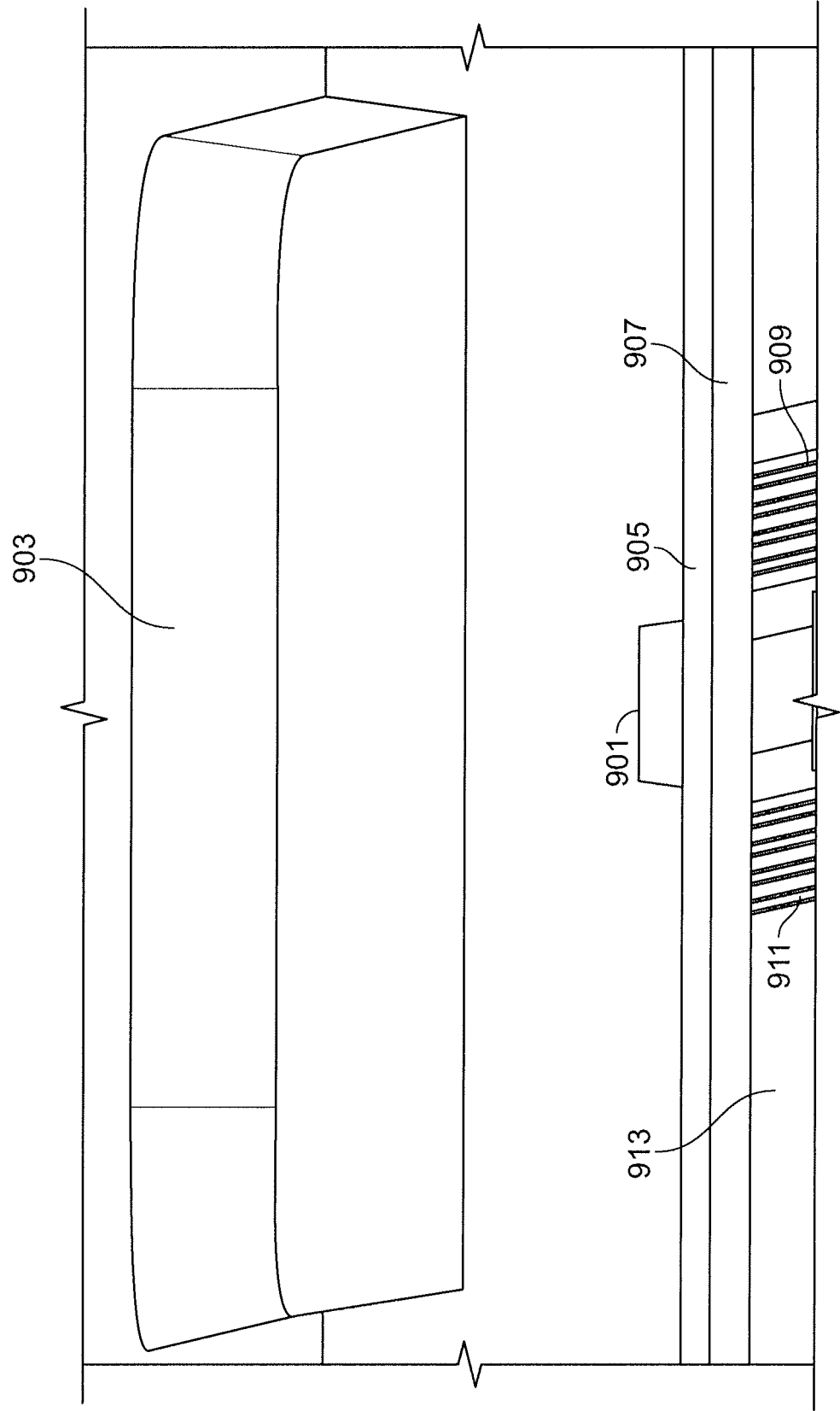


FIG. 48

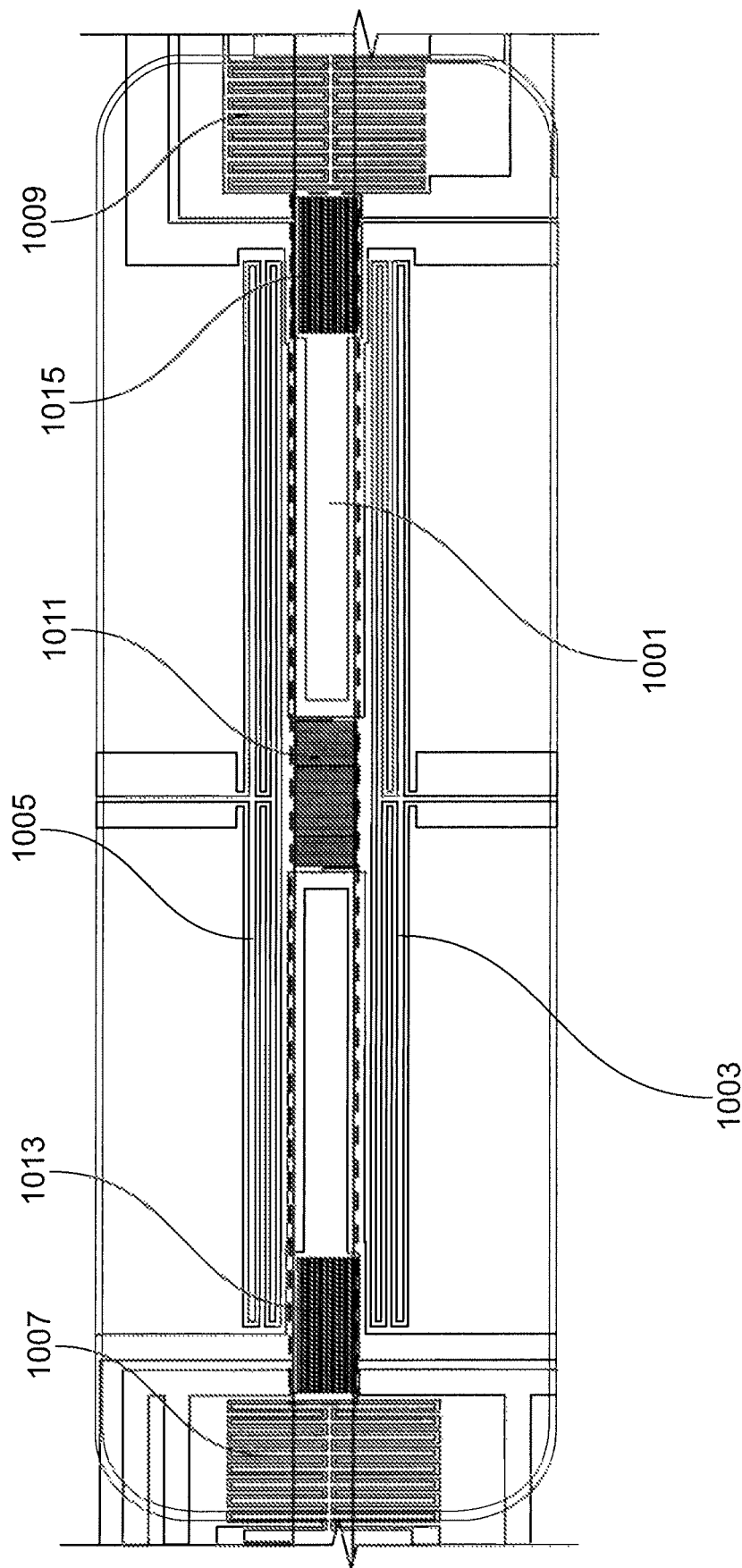


FIG. 49A

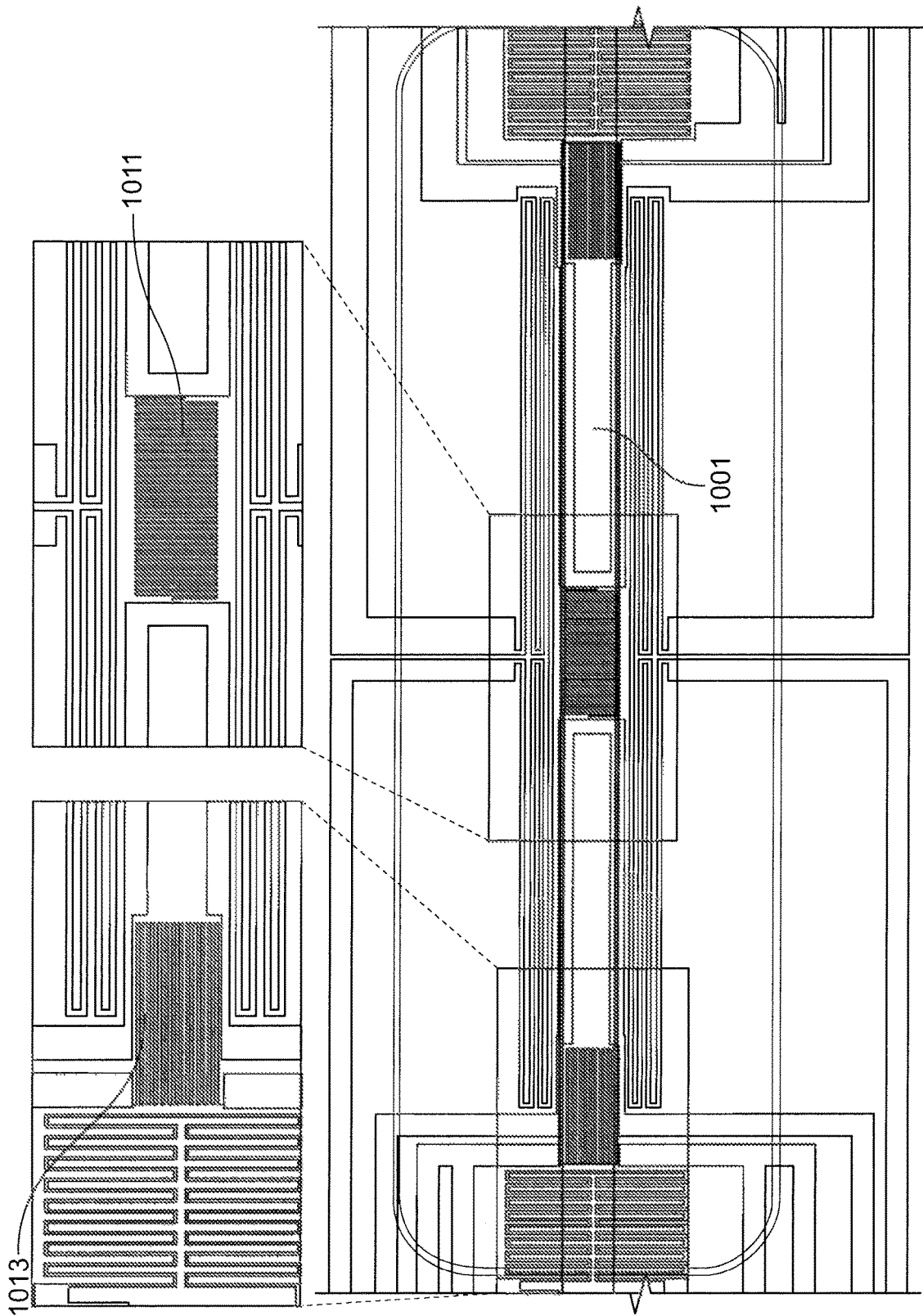


FIG. 49B

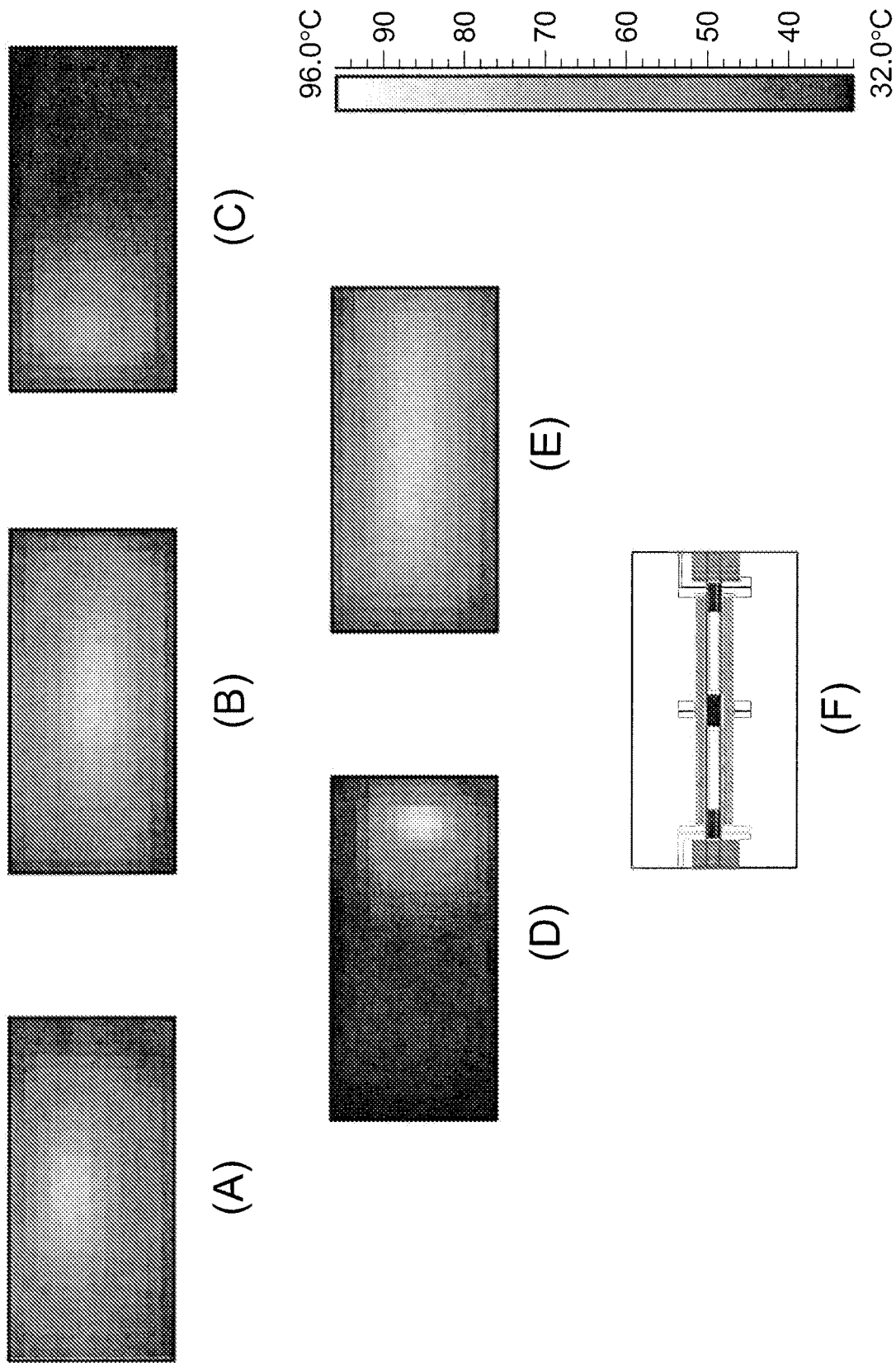
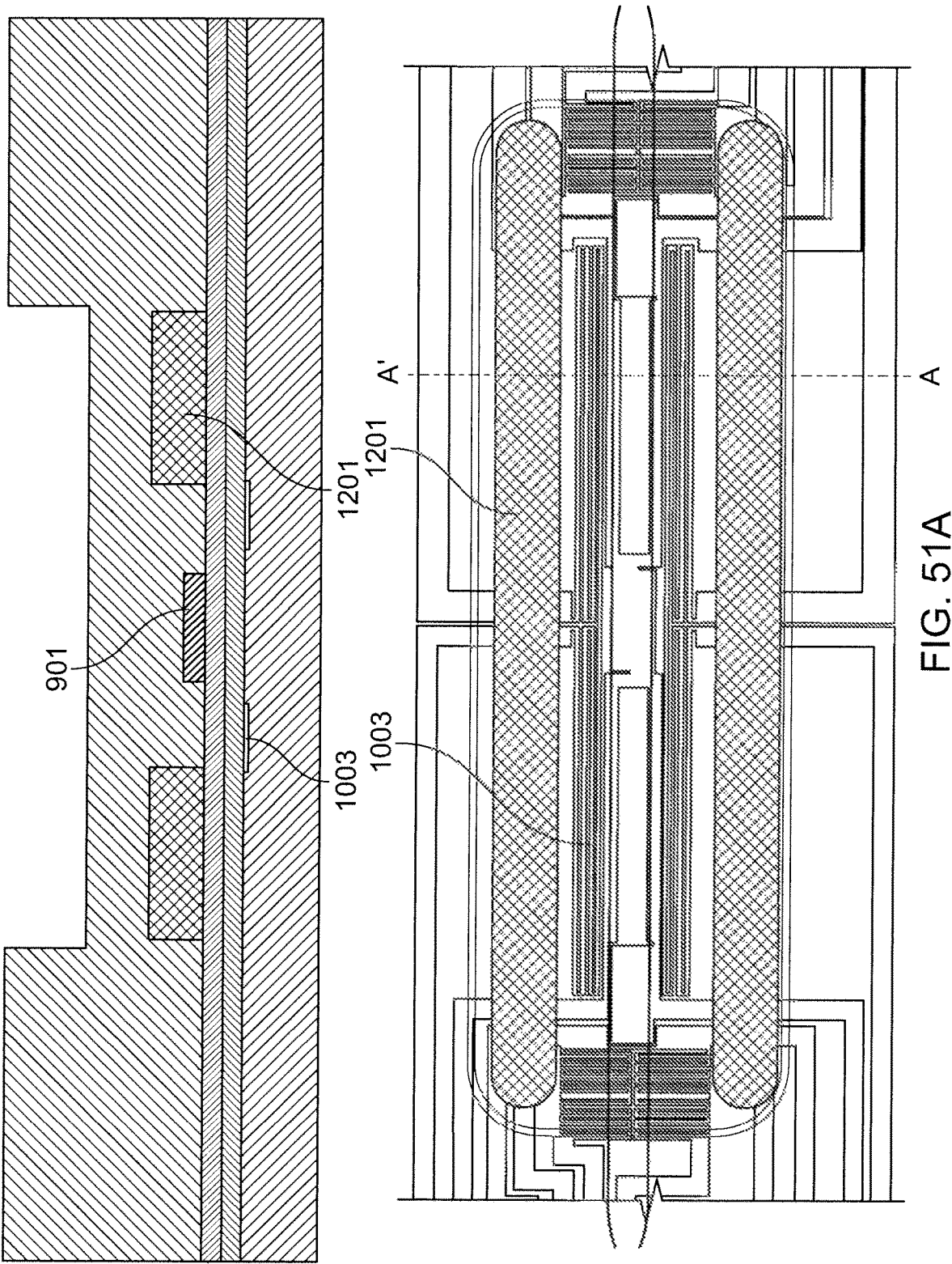


FIG. 50



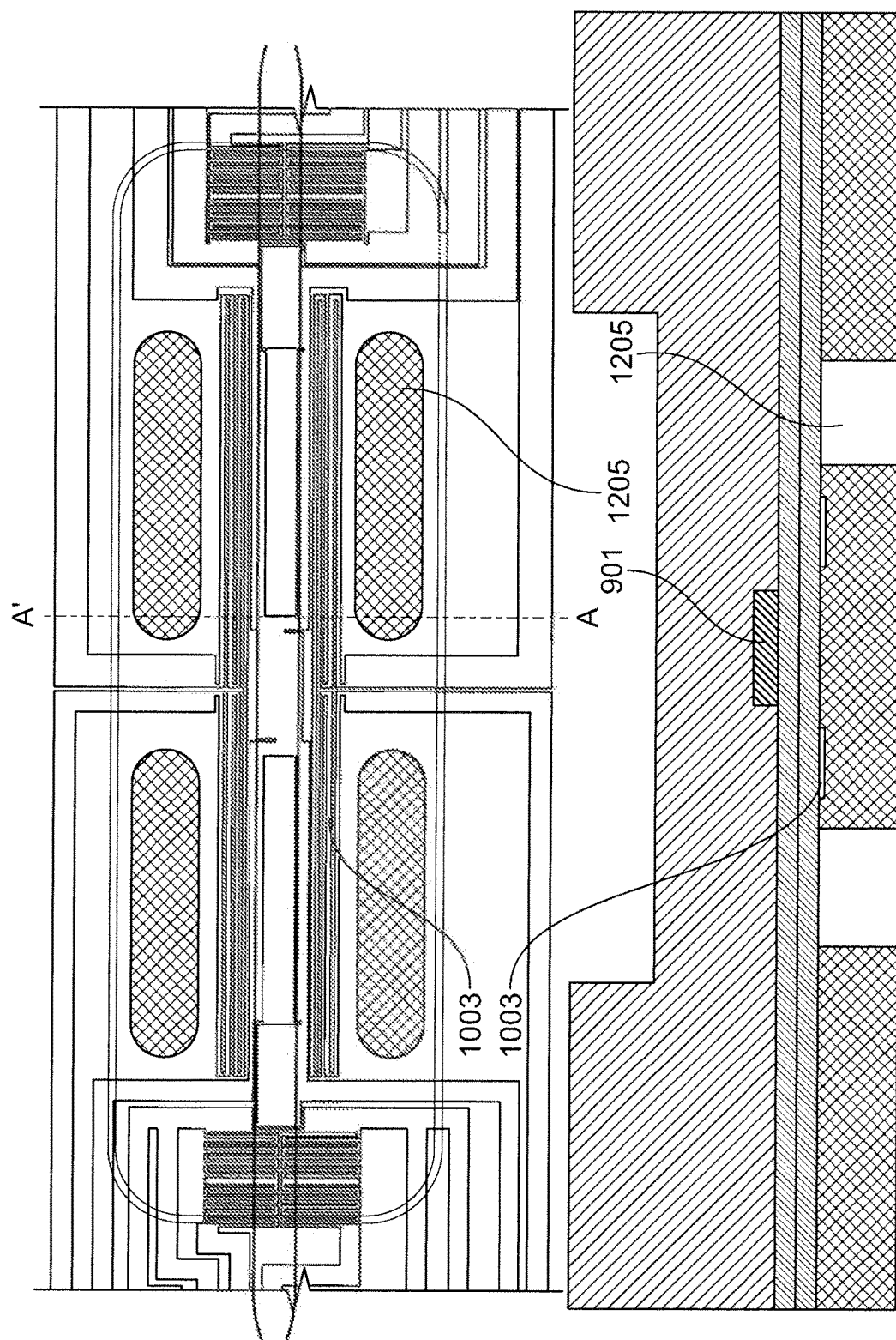


FIG. 51B

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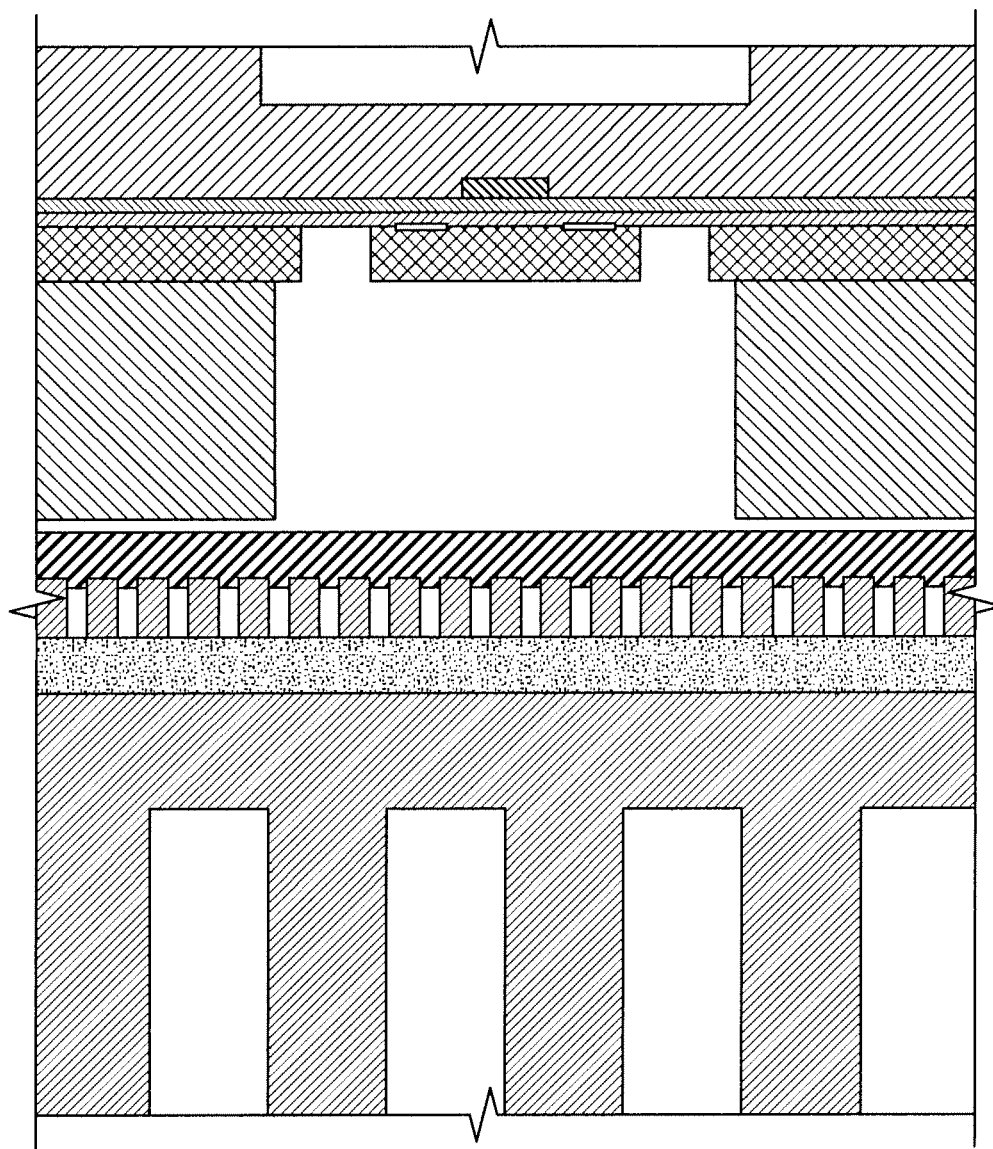


FIG. 51C

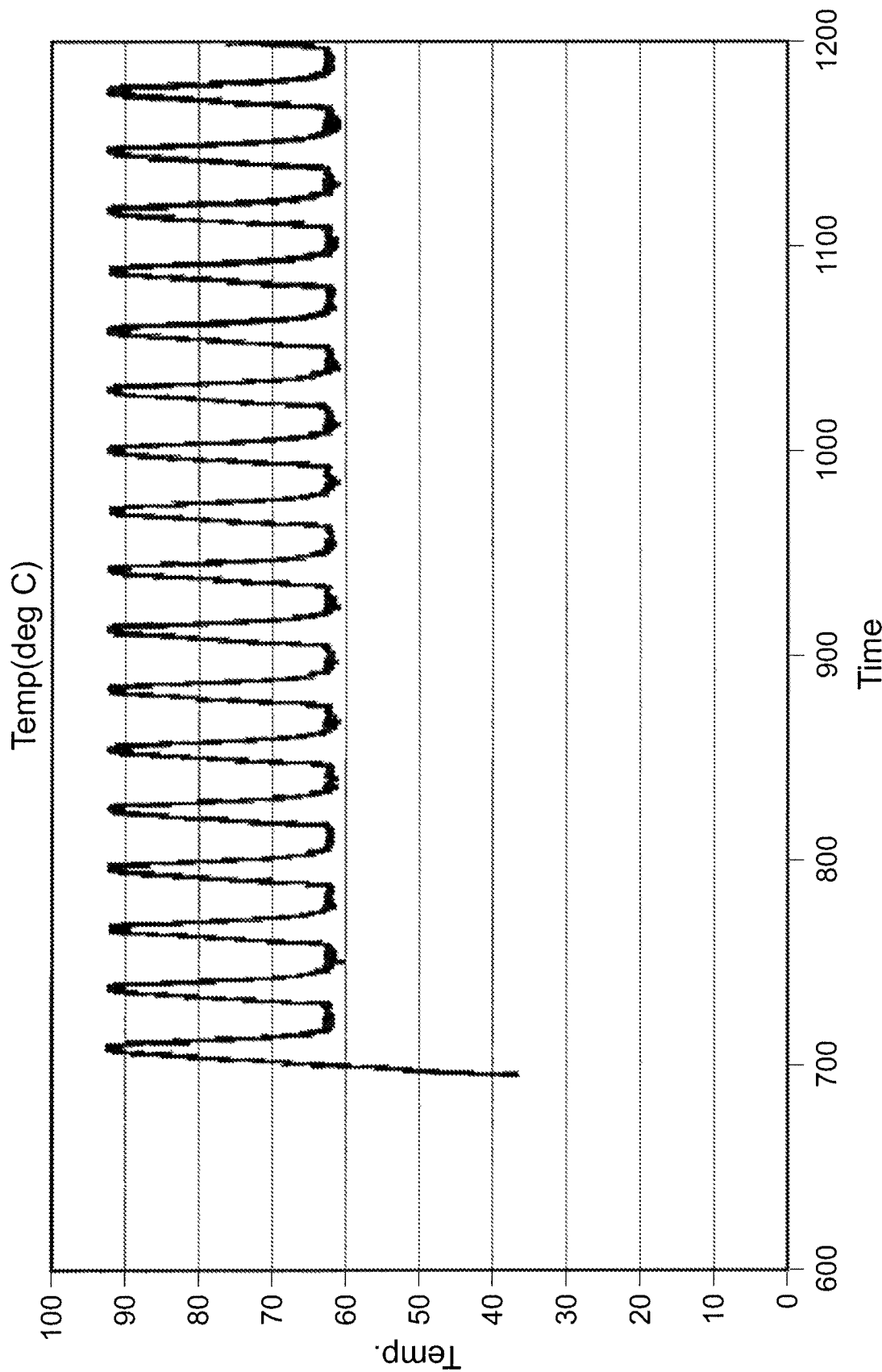


FIG. 52

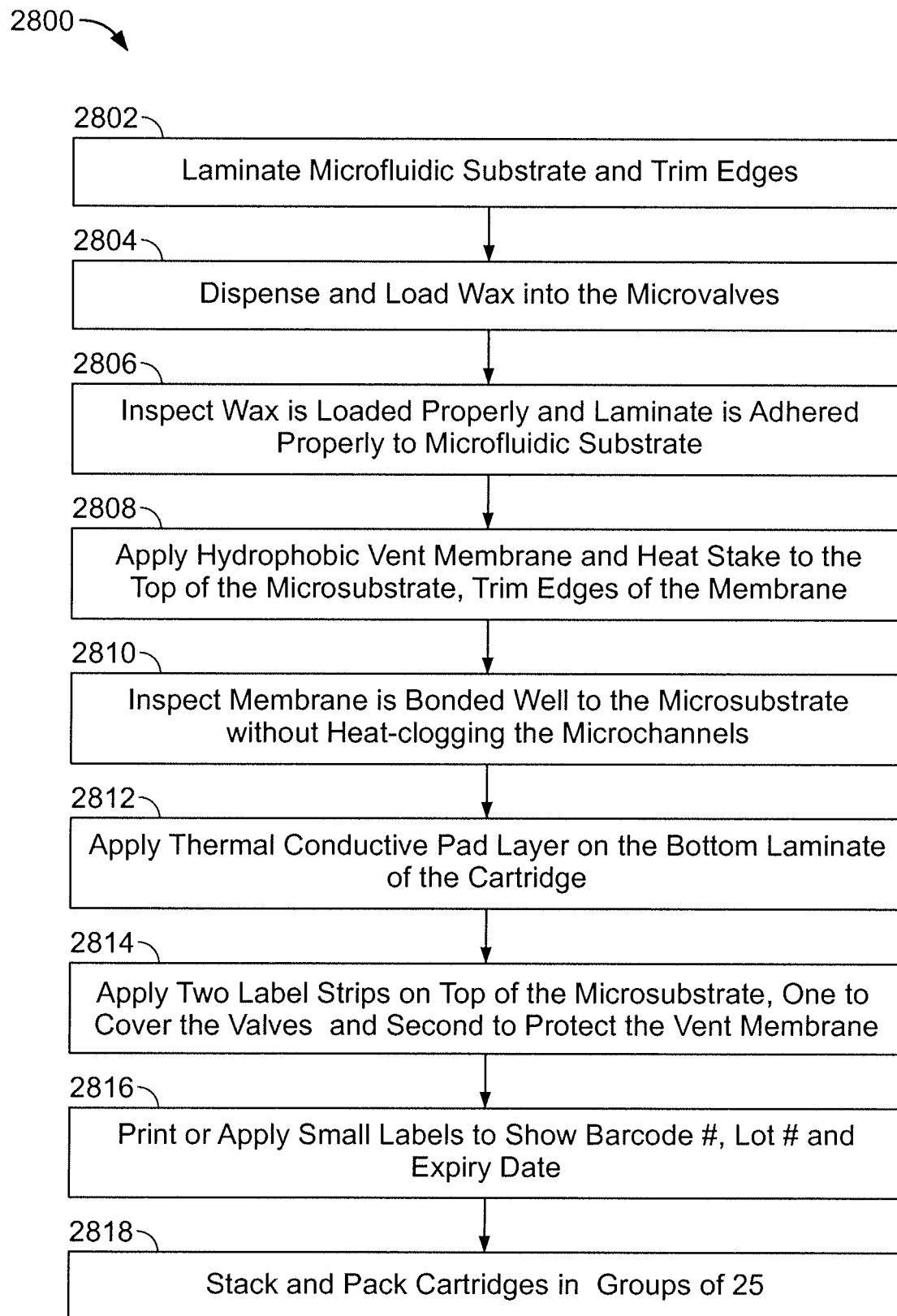


FIG. 53

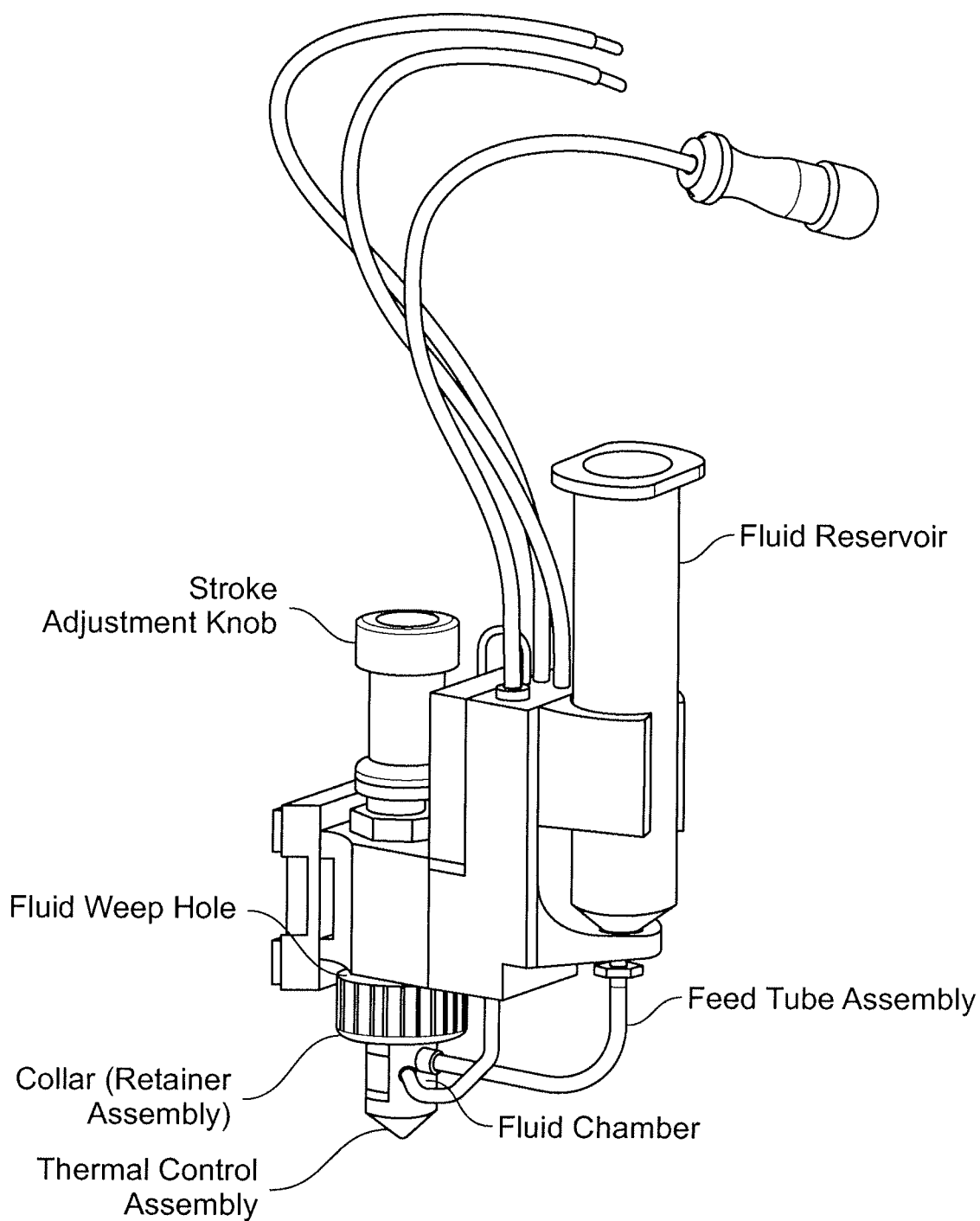


FIG. 54A

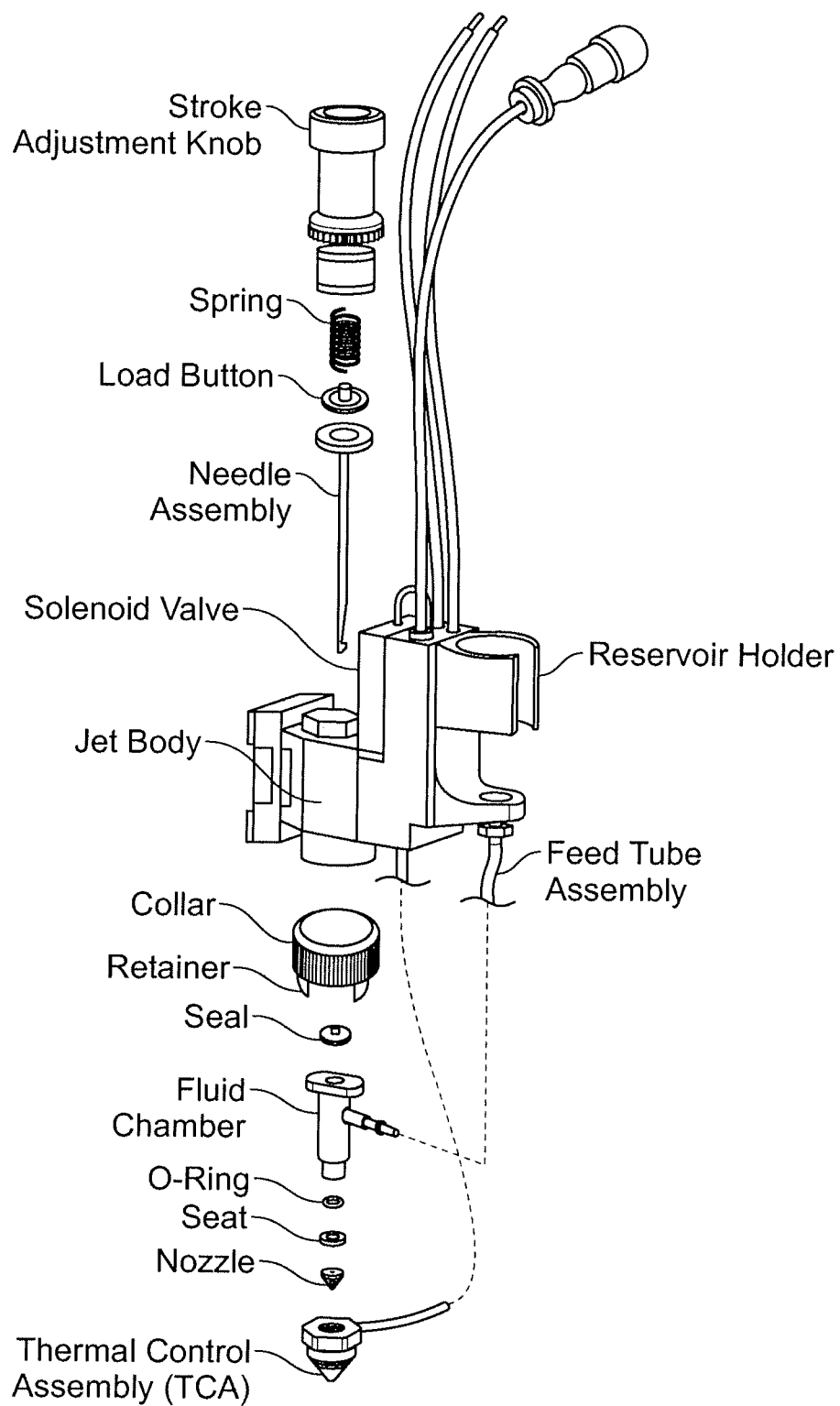


FIG. 54B

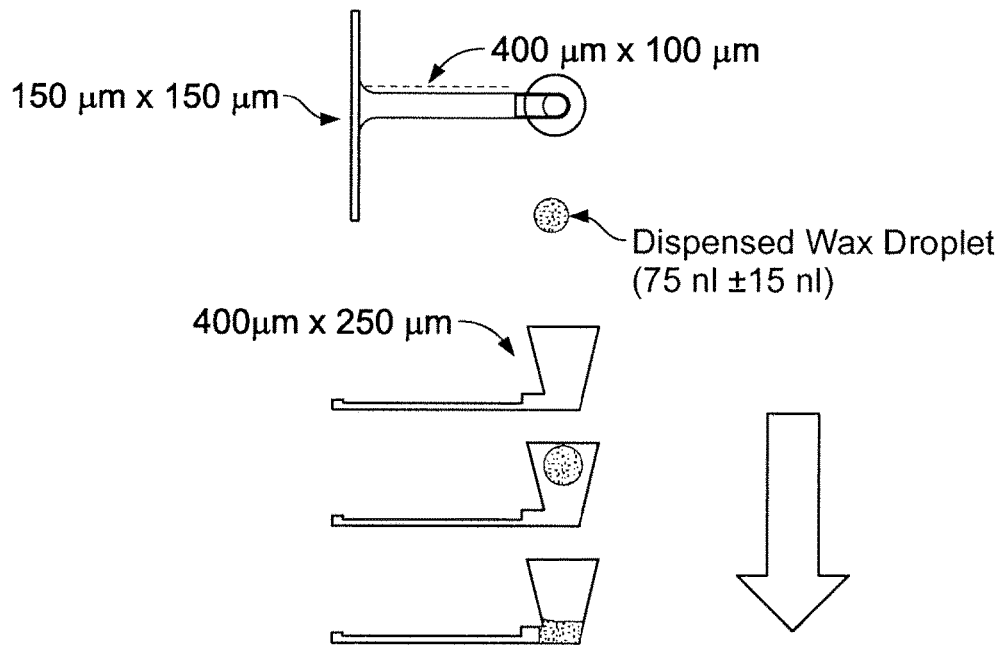


FIG. 55A

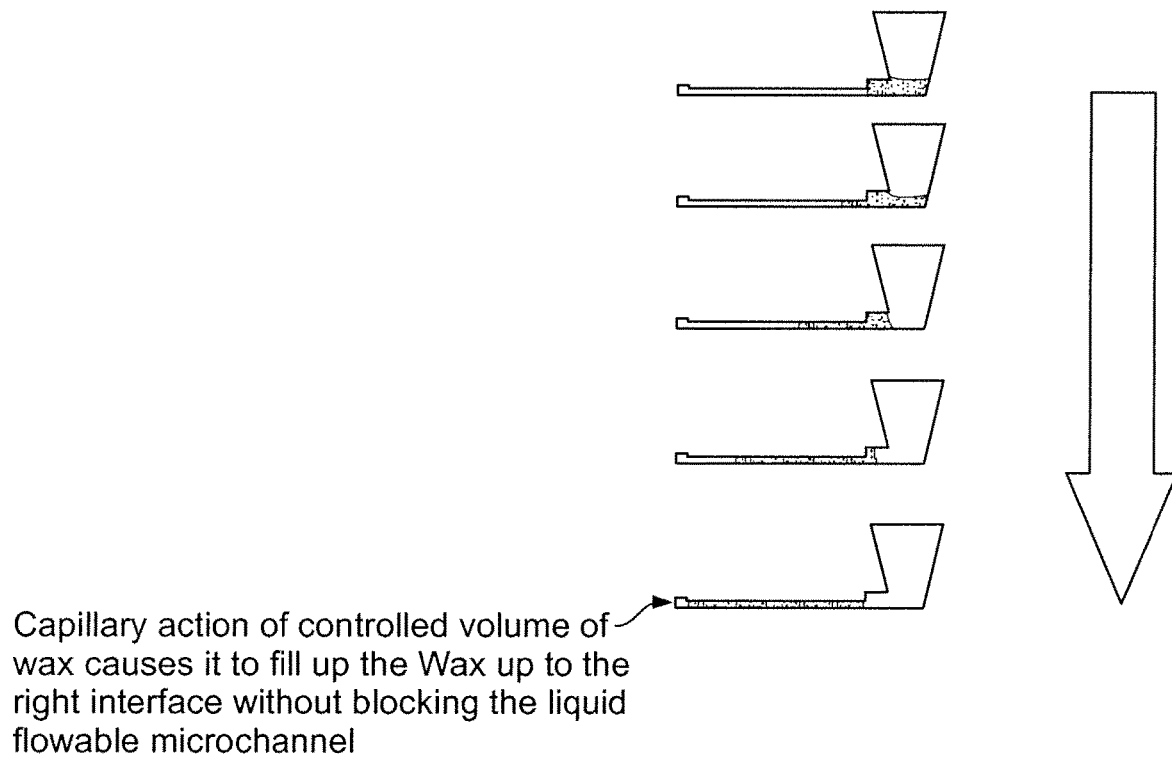


FIG. 55B

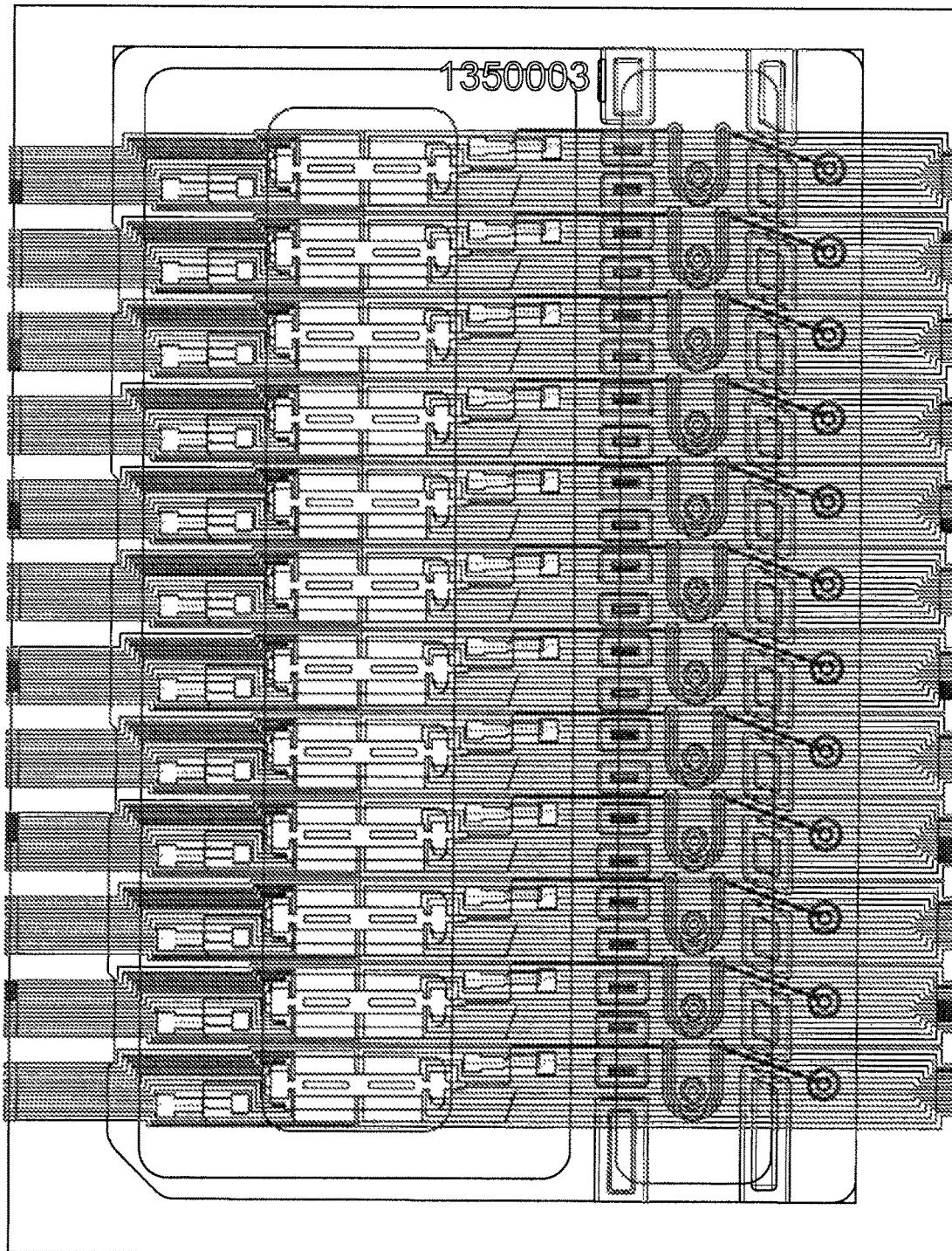


FIG. 56

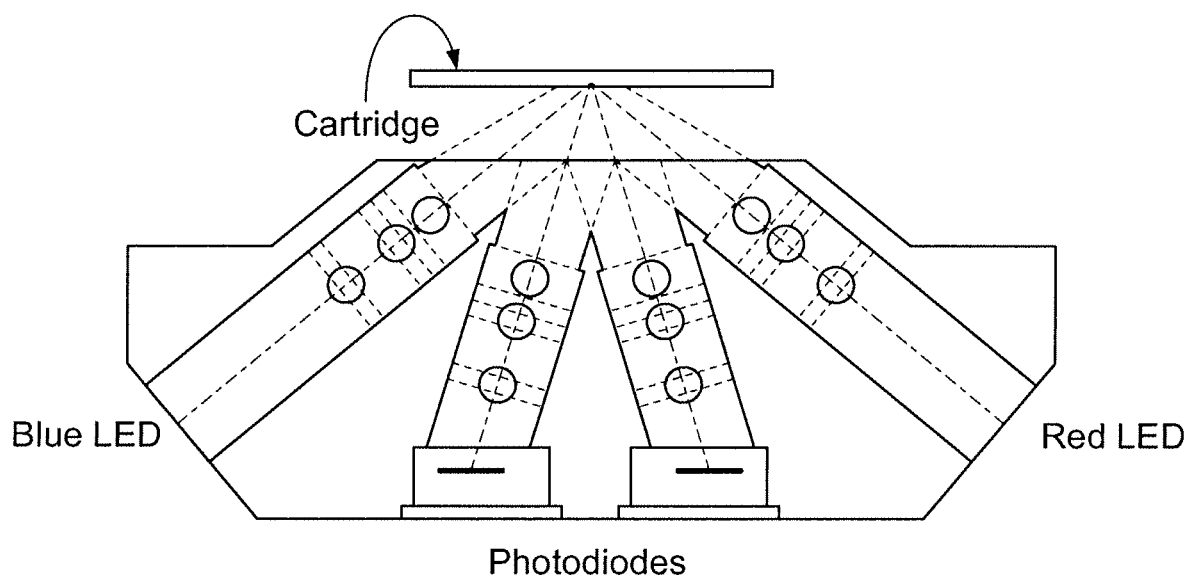


FIG. 57

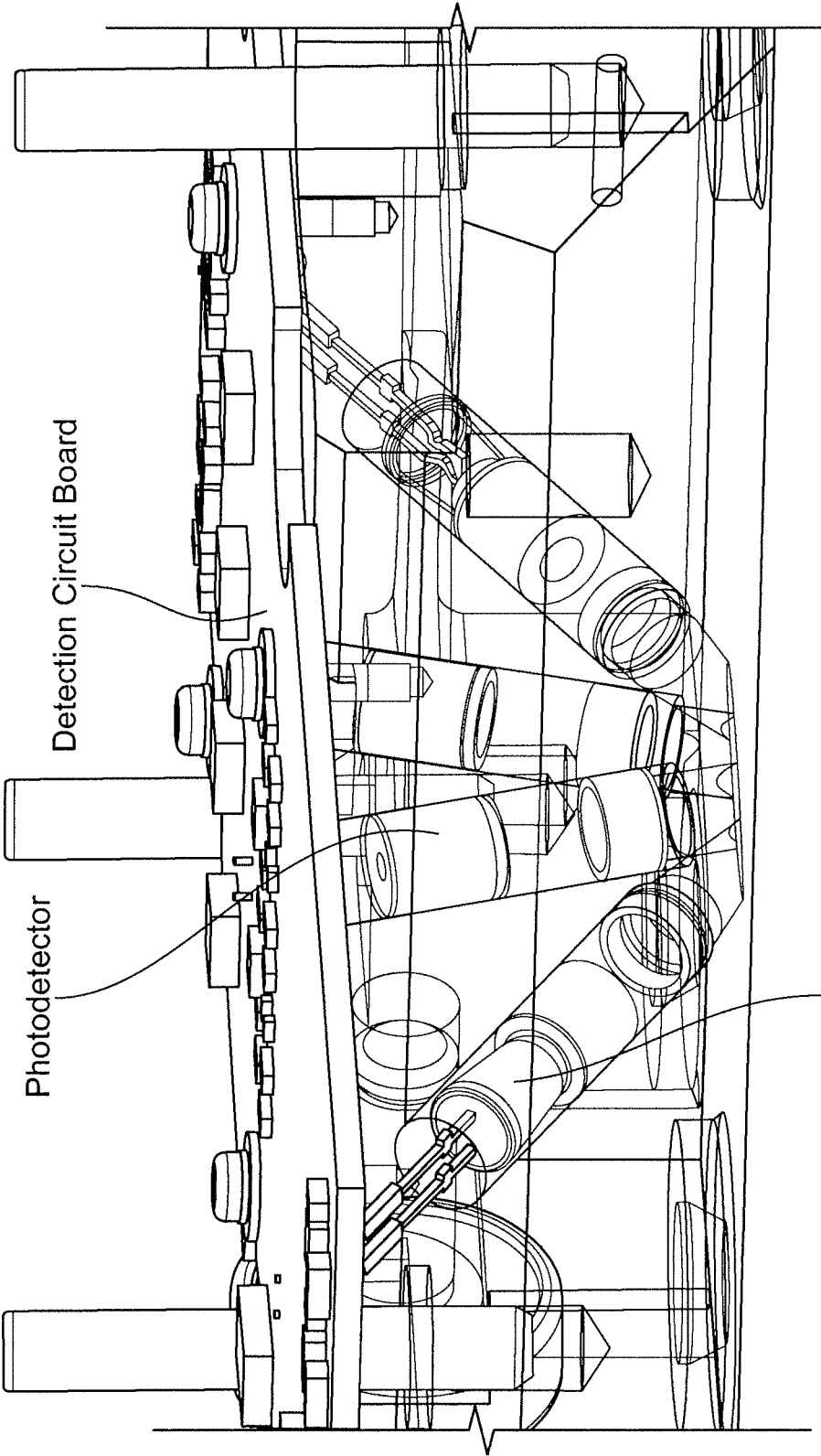


FIG. 58

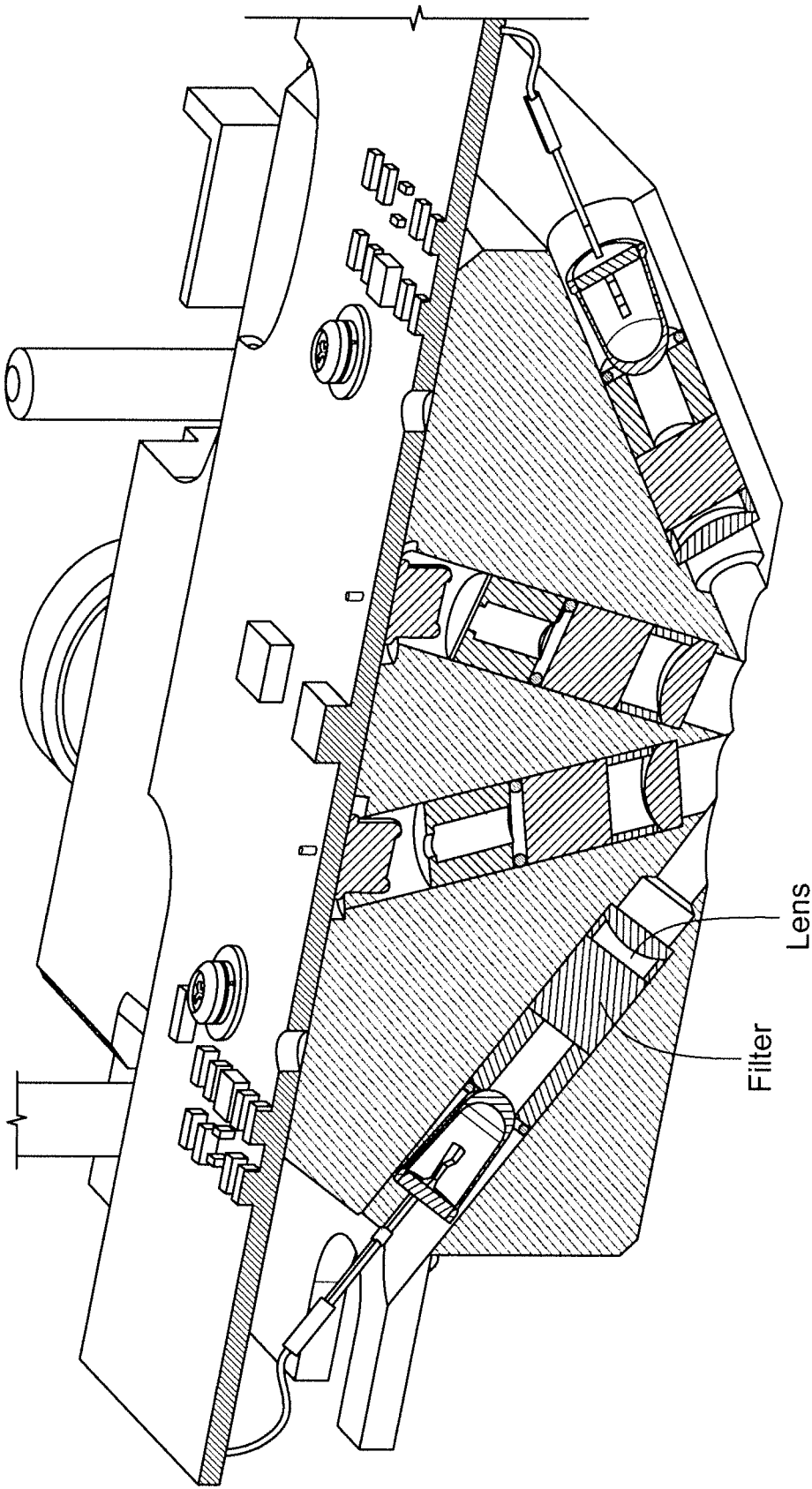


FIG. 59

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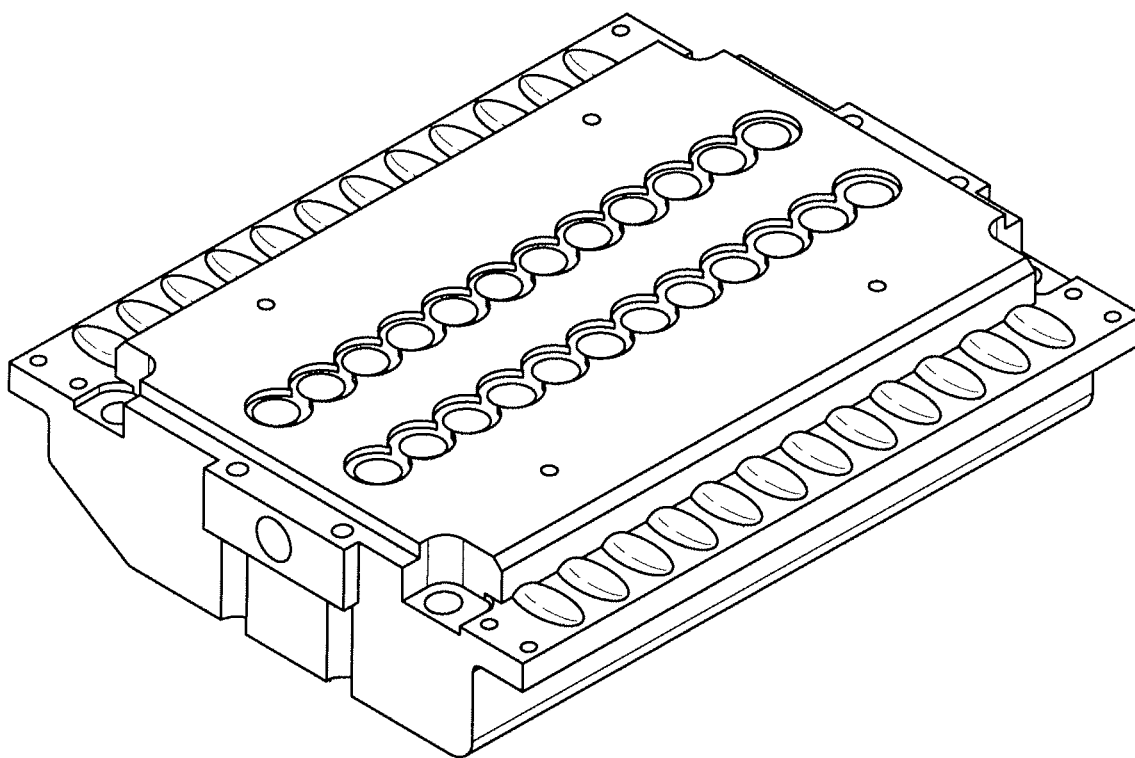


FIG. 60

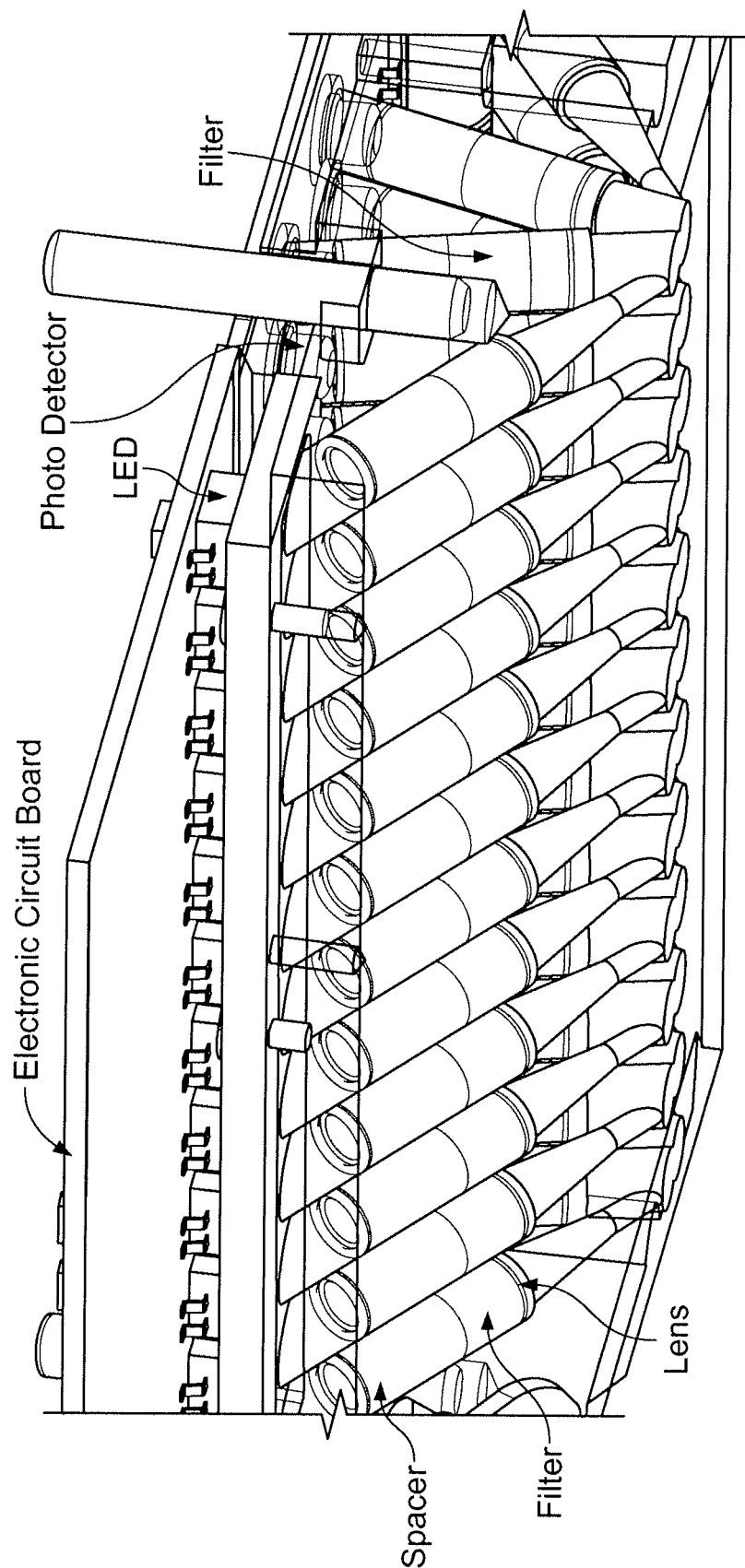


FIG. 61

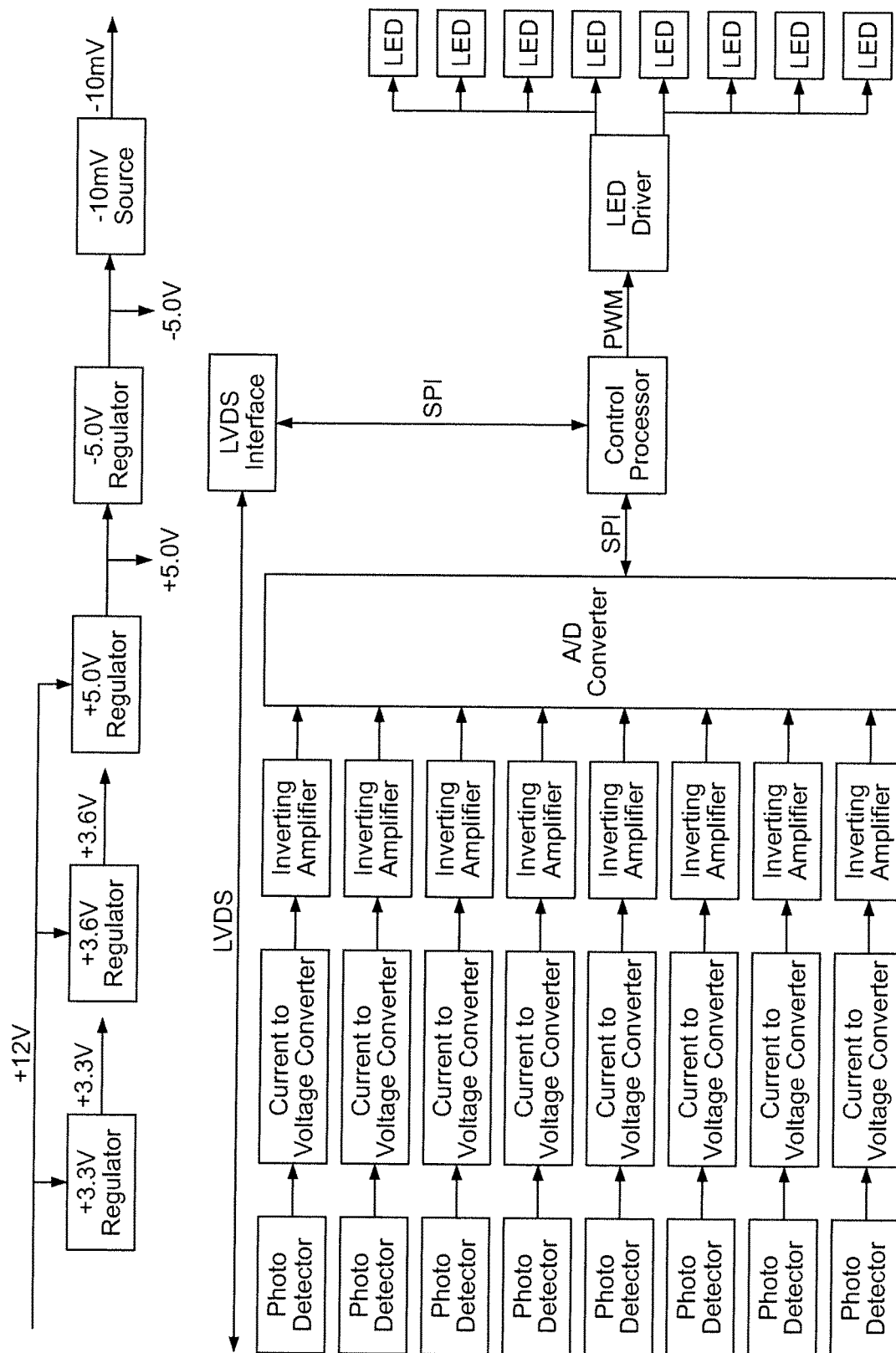


FIG. 62

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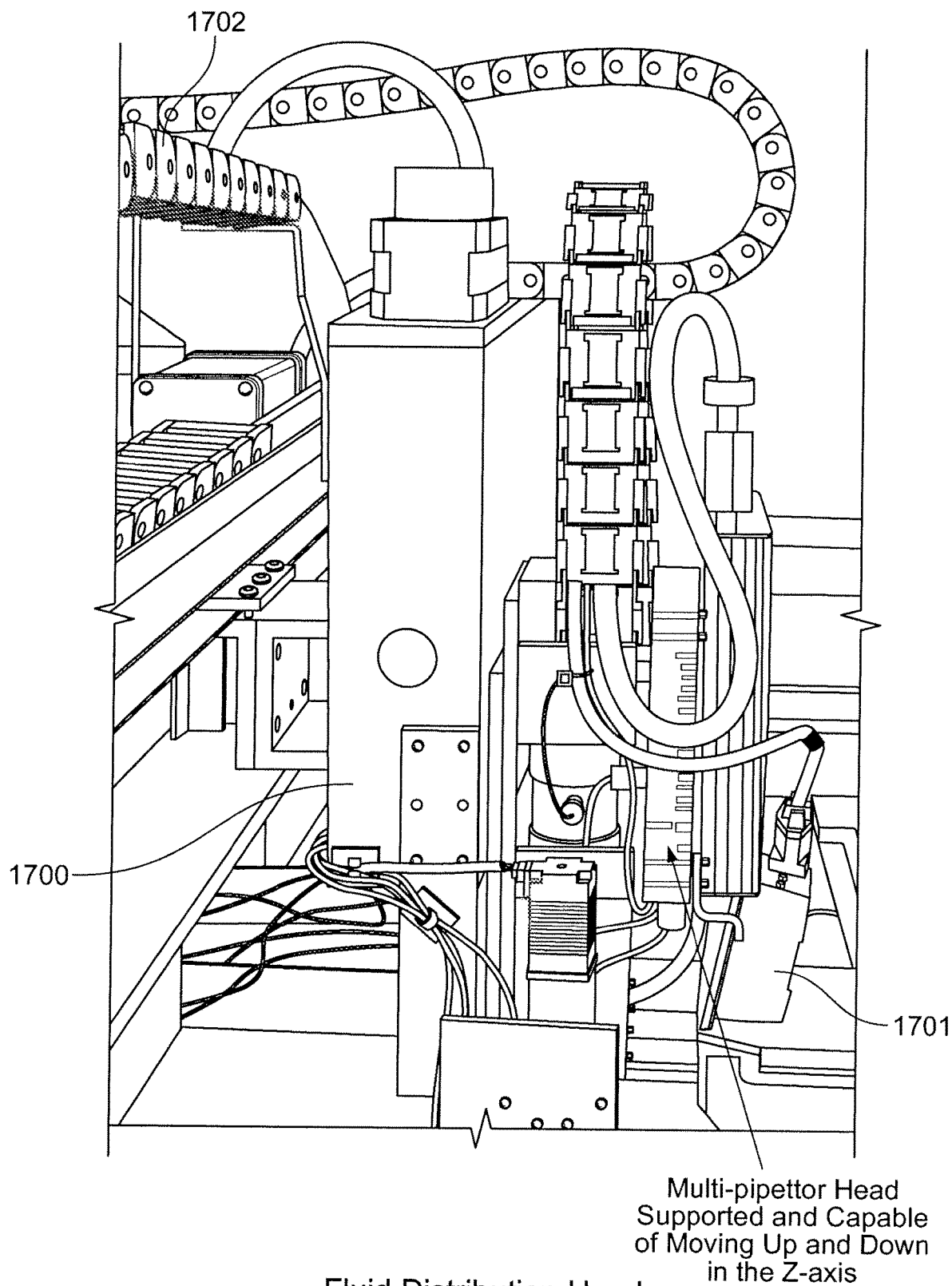


FIG. 63

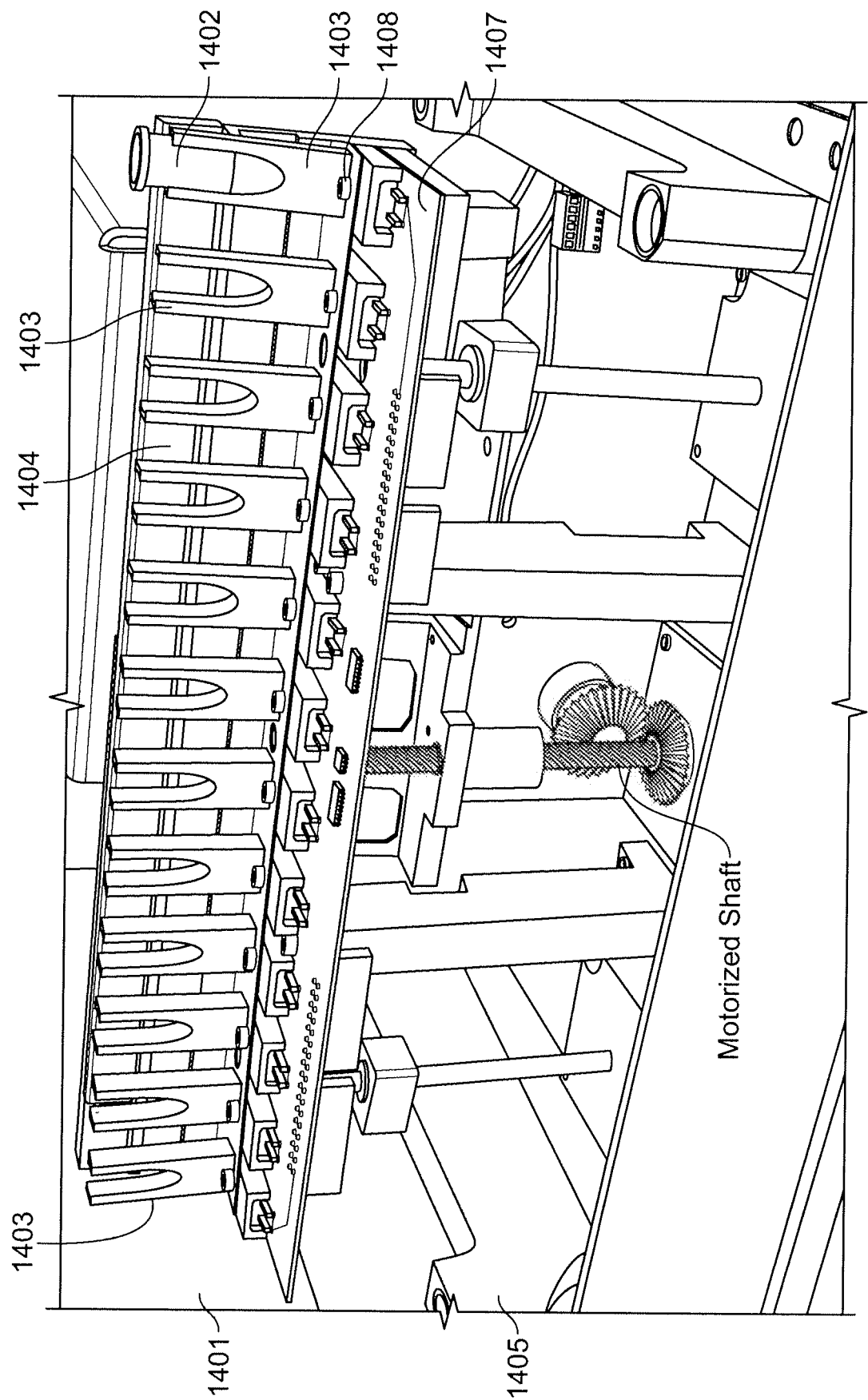


FIG. 64

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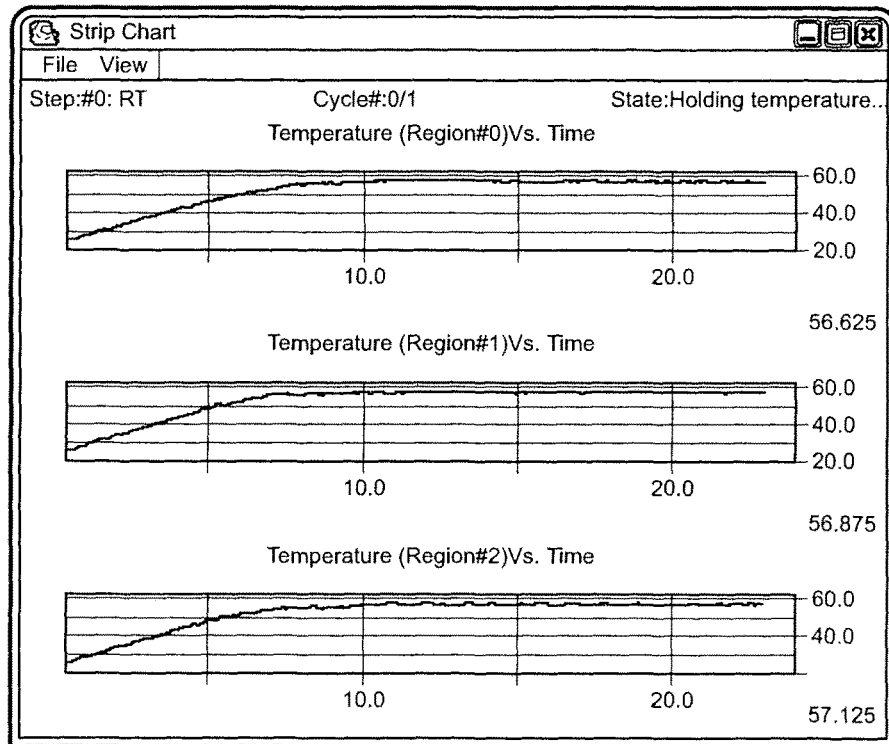


FIG. 65A

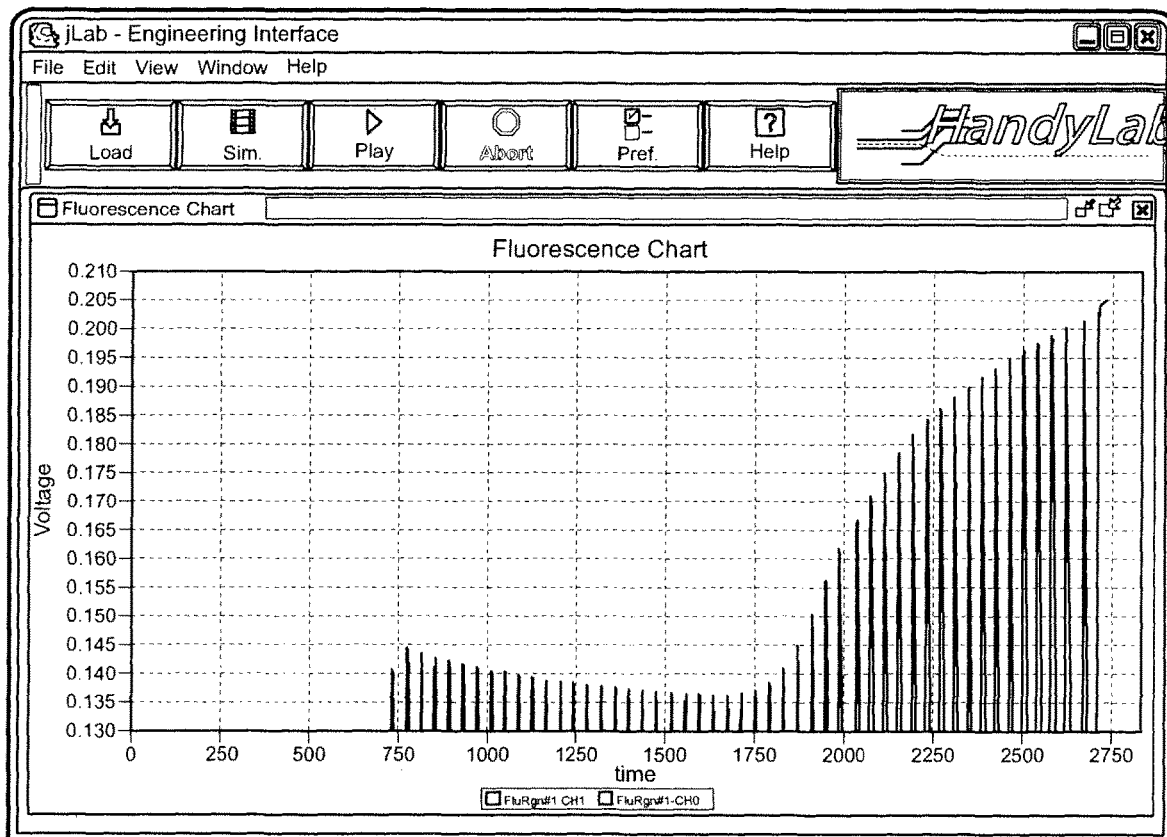


FIG. 65B

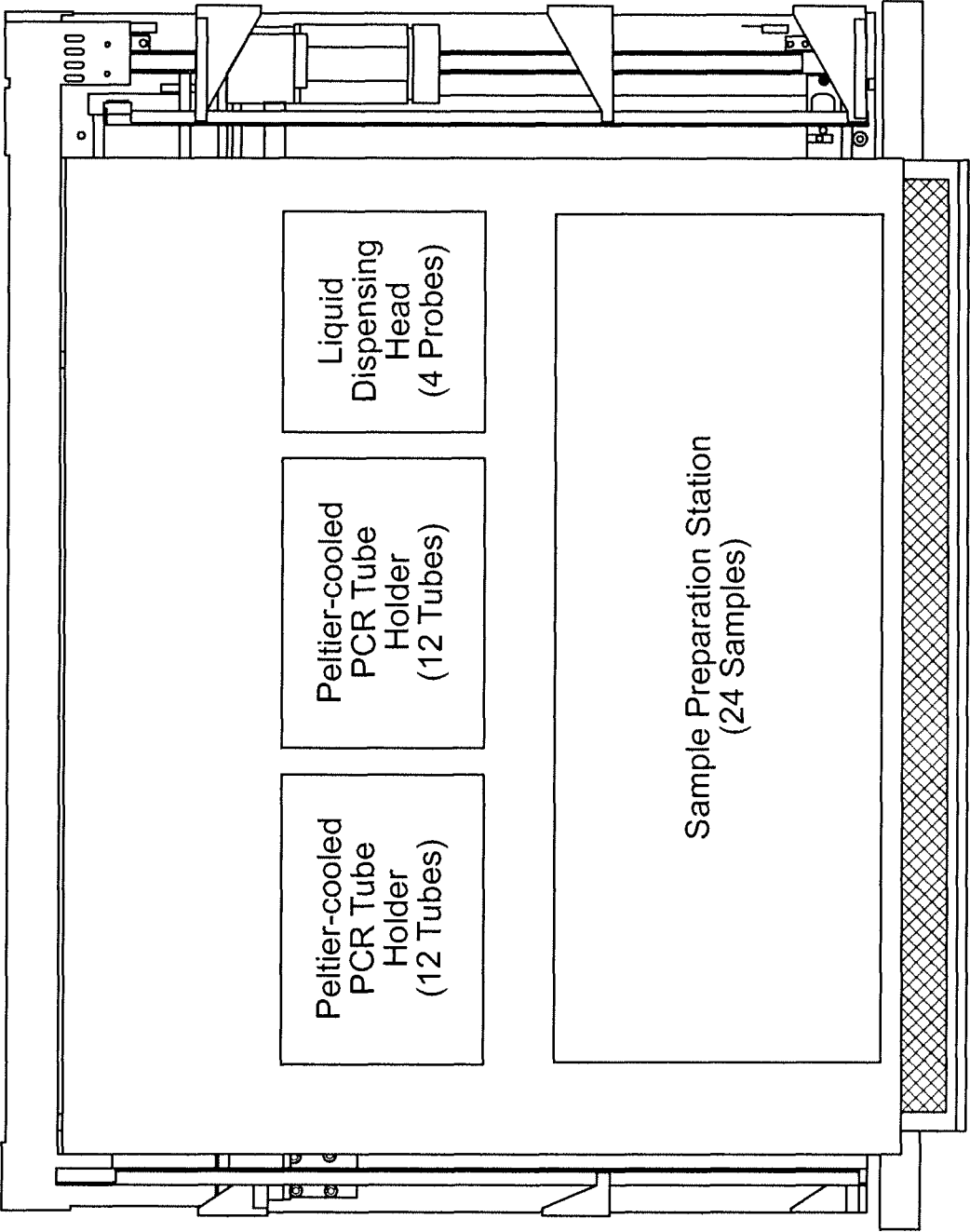
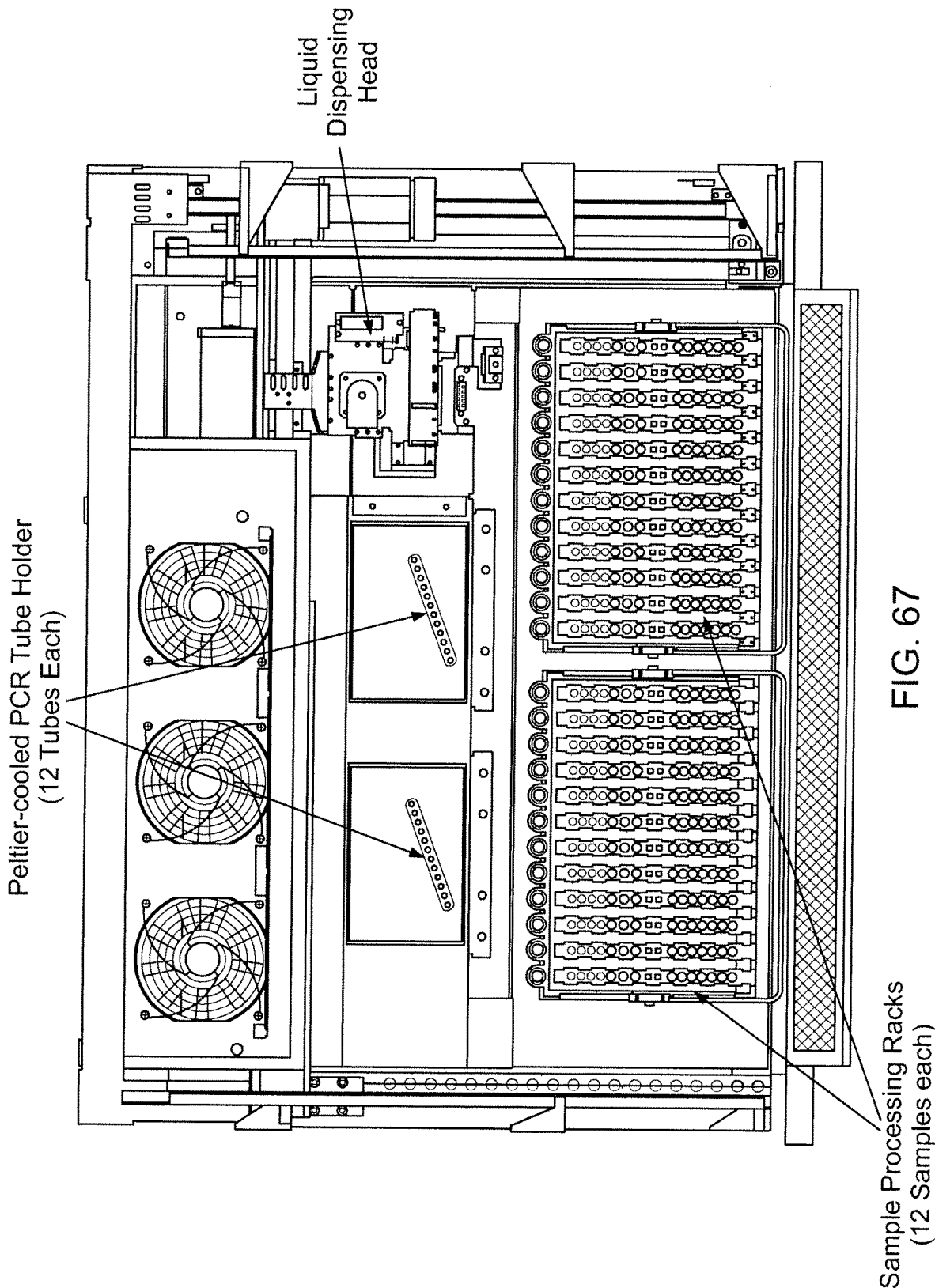


FIG. 66



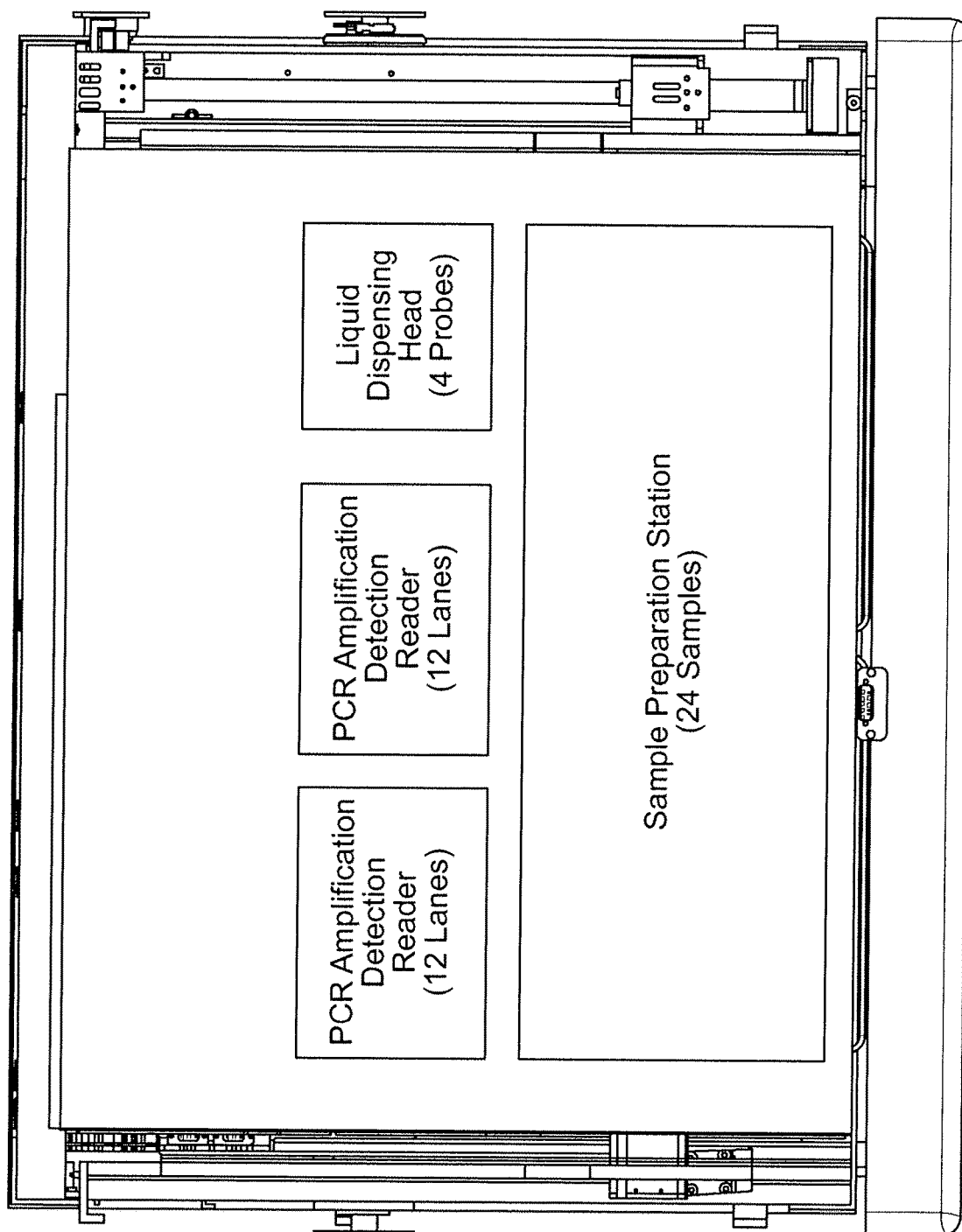


FIG. 68

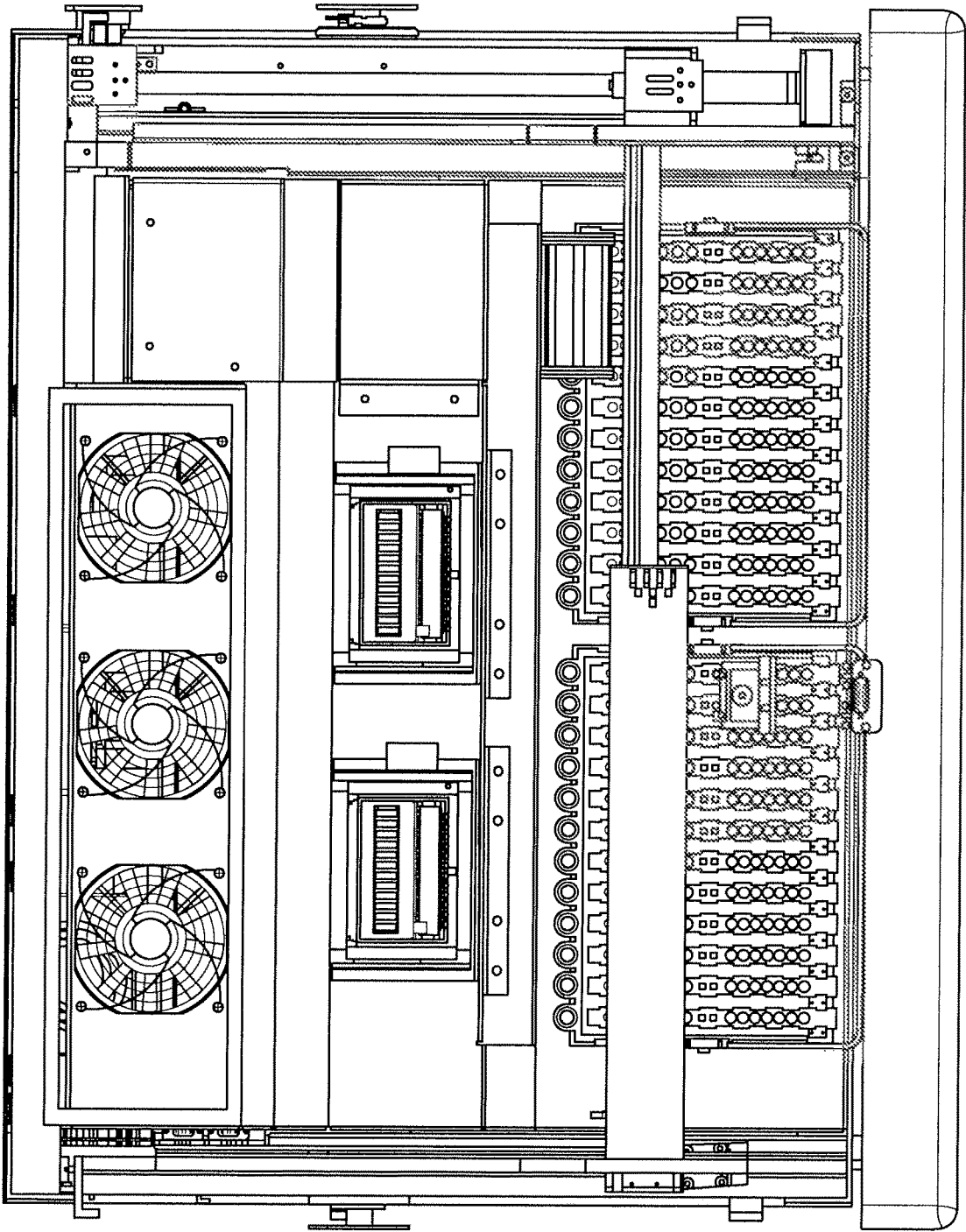


FIG. 69

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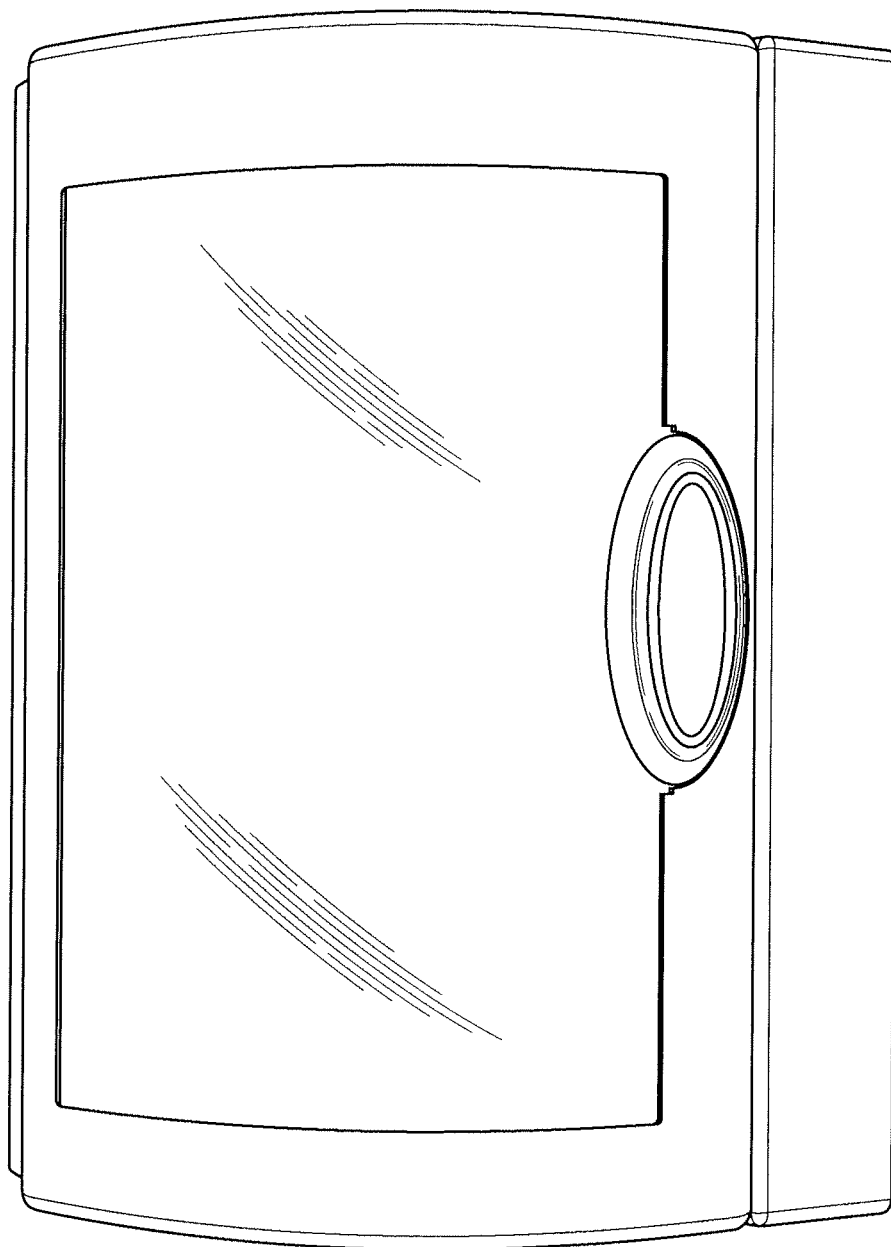


FIG. 70

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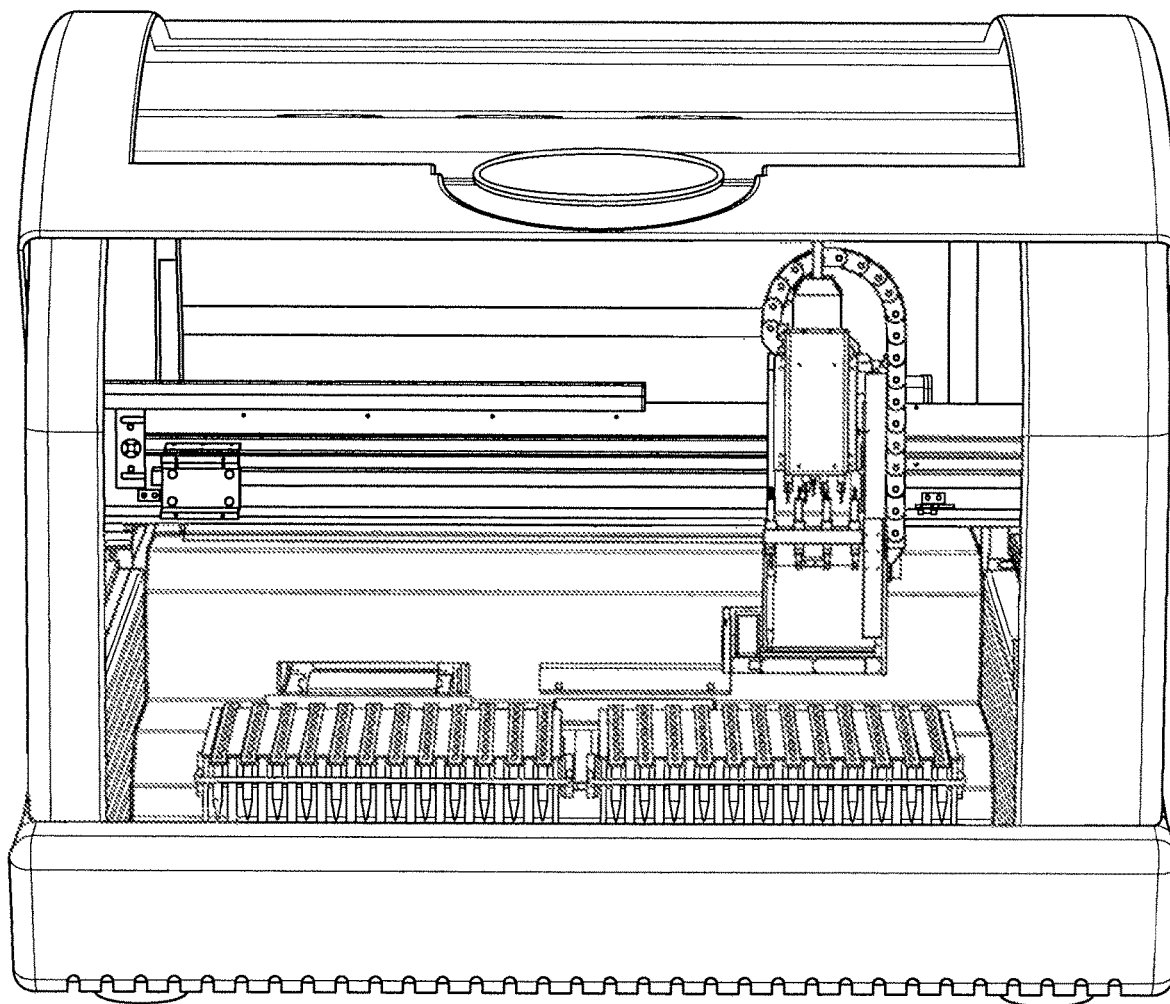
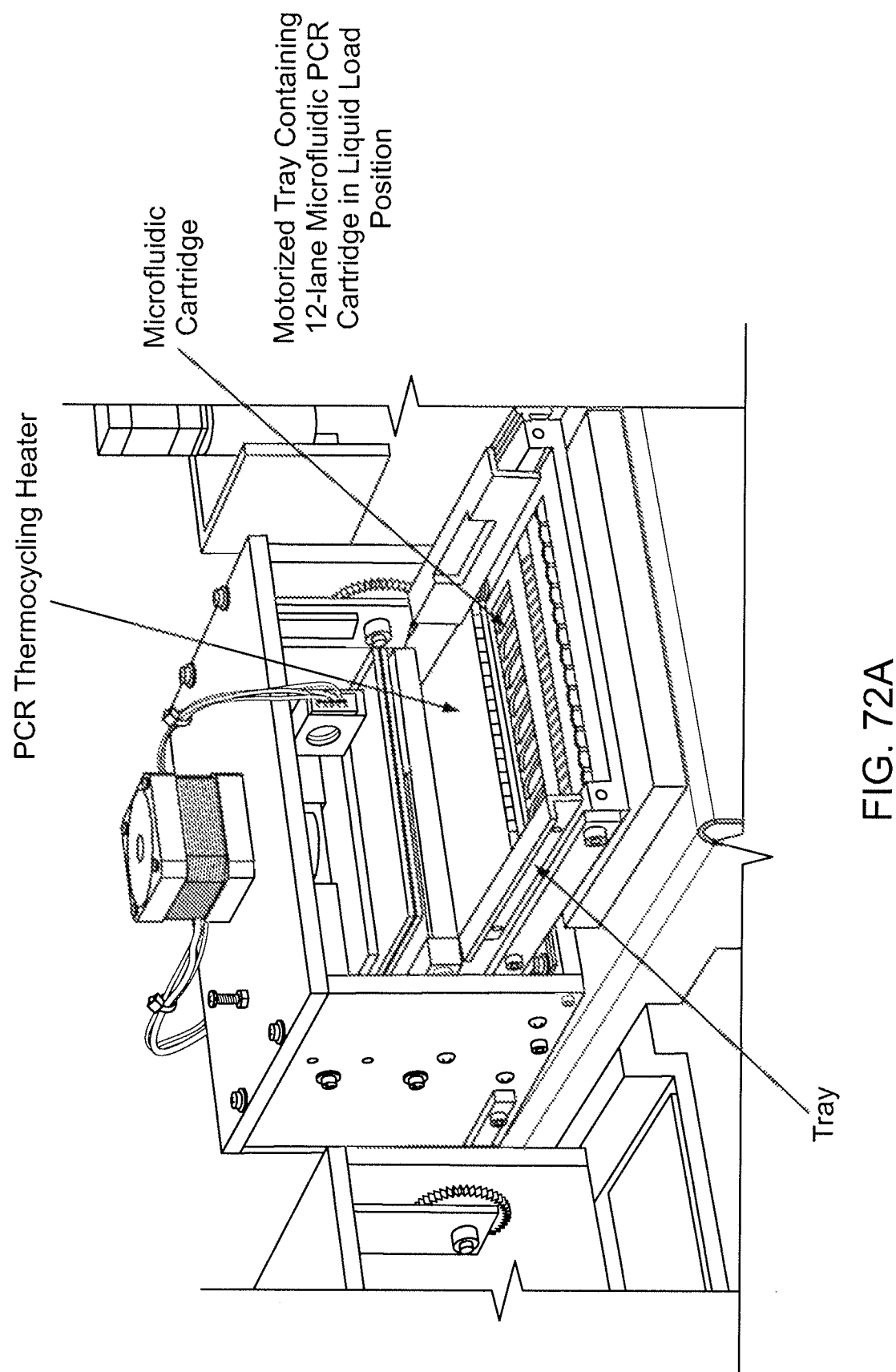
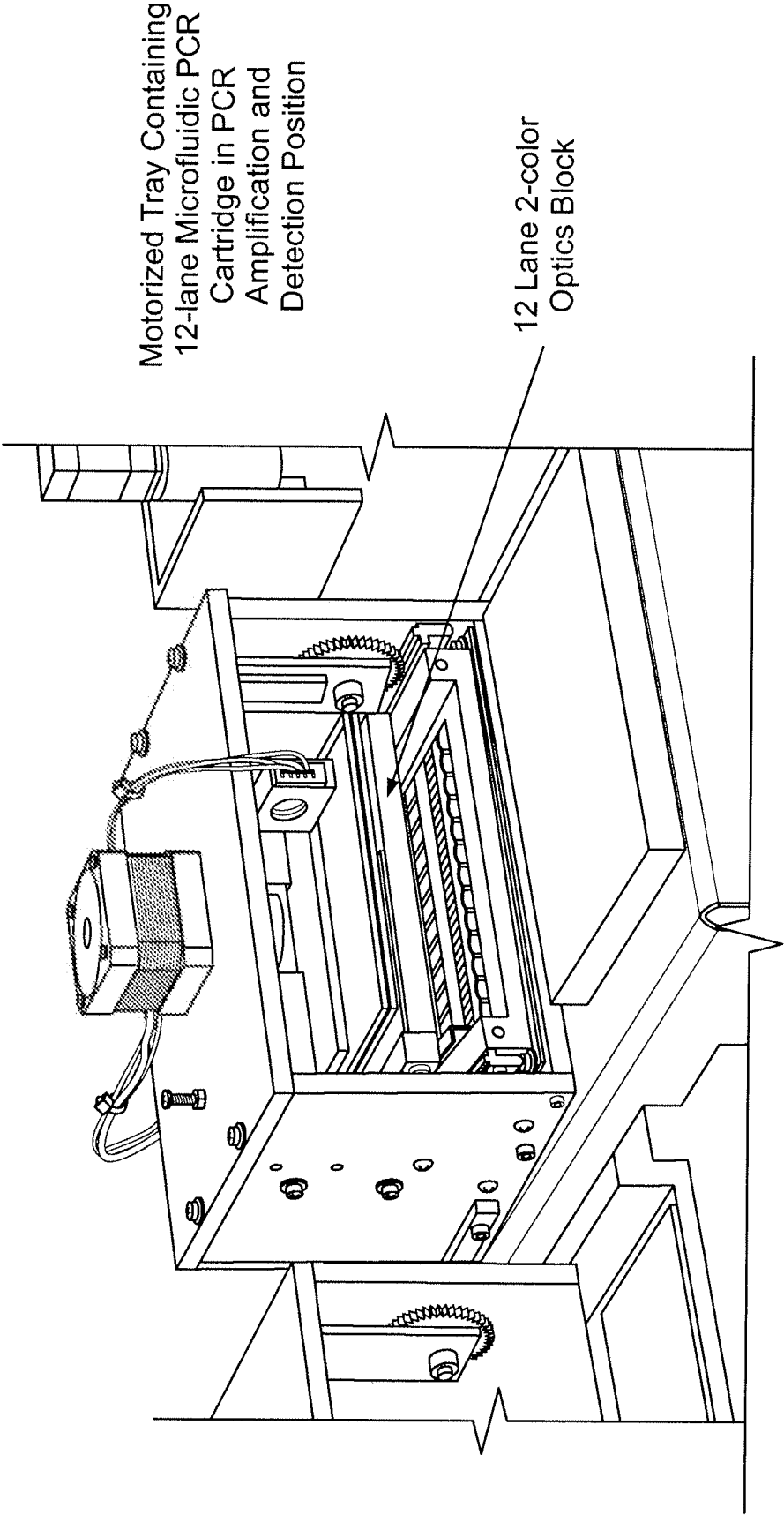


FIG. 71





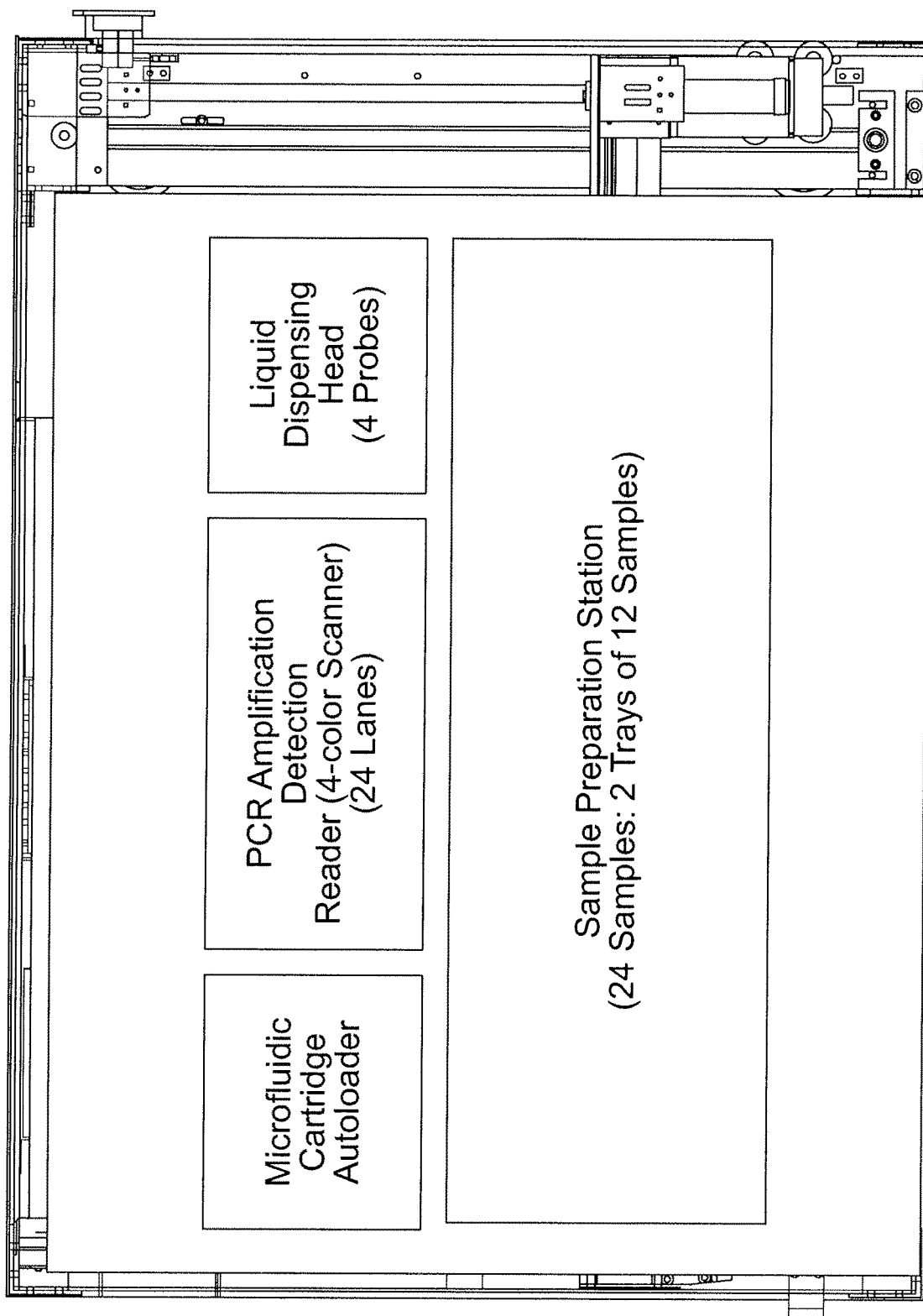


FIG. 73

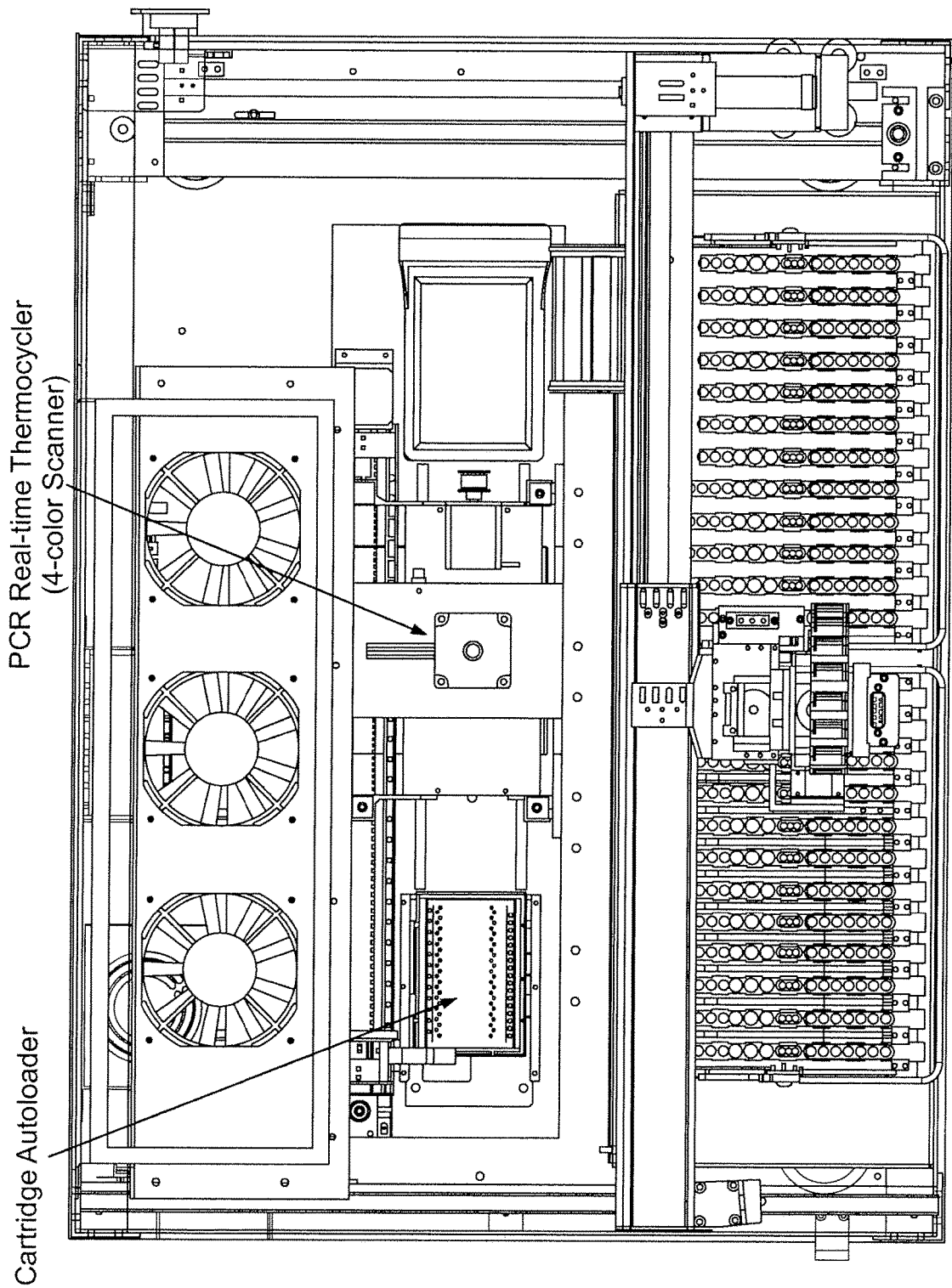


FIG. 74

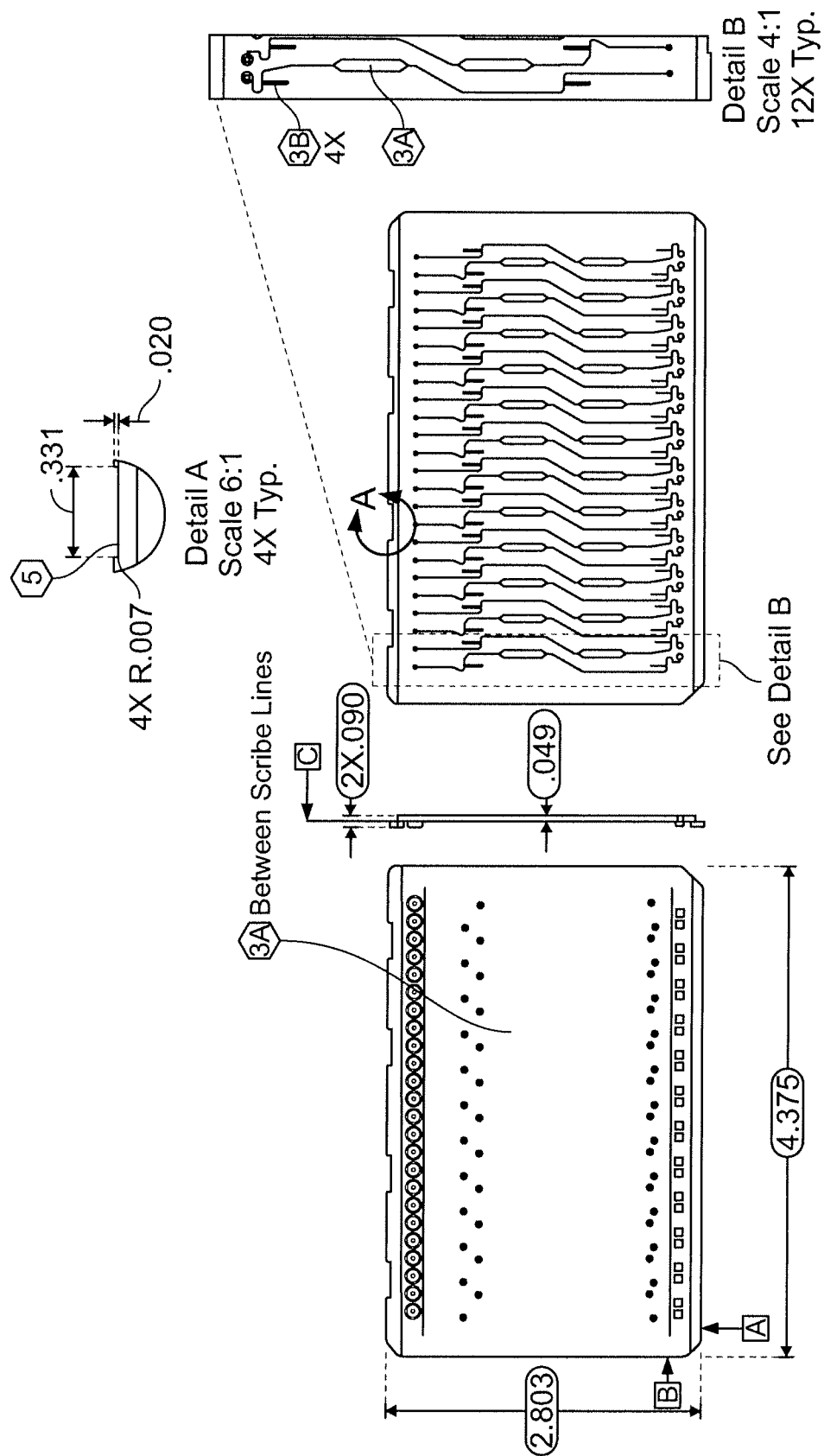


FIG. 75

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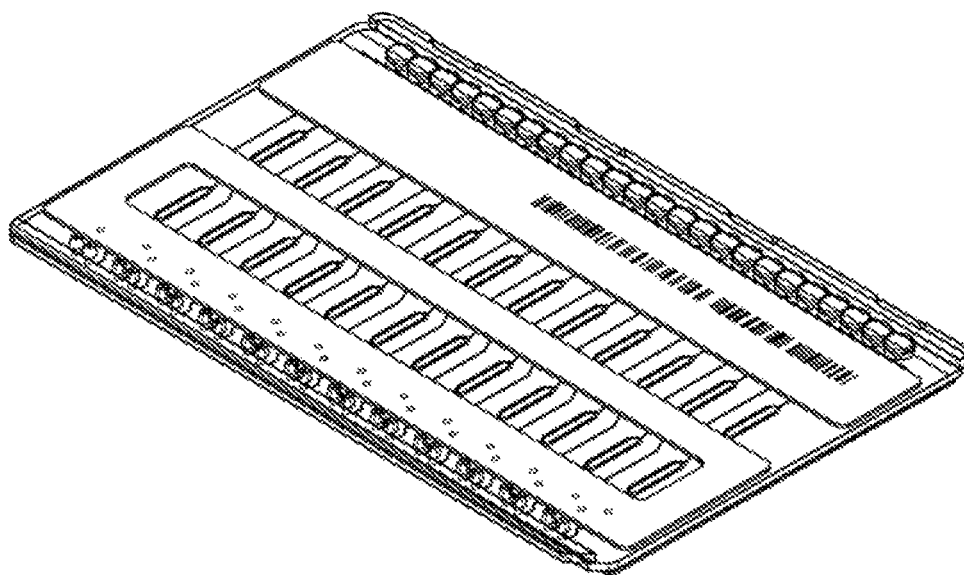


FIG. 76

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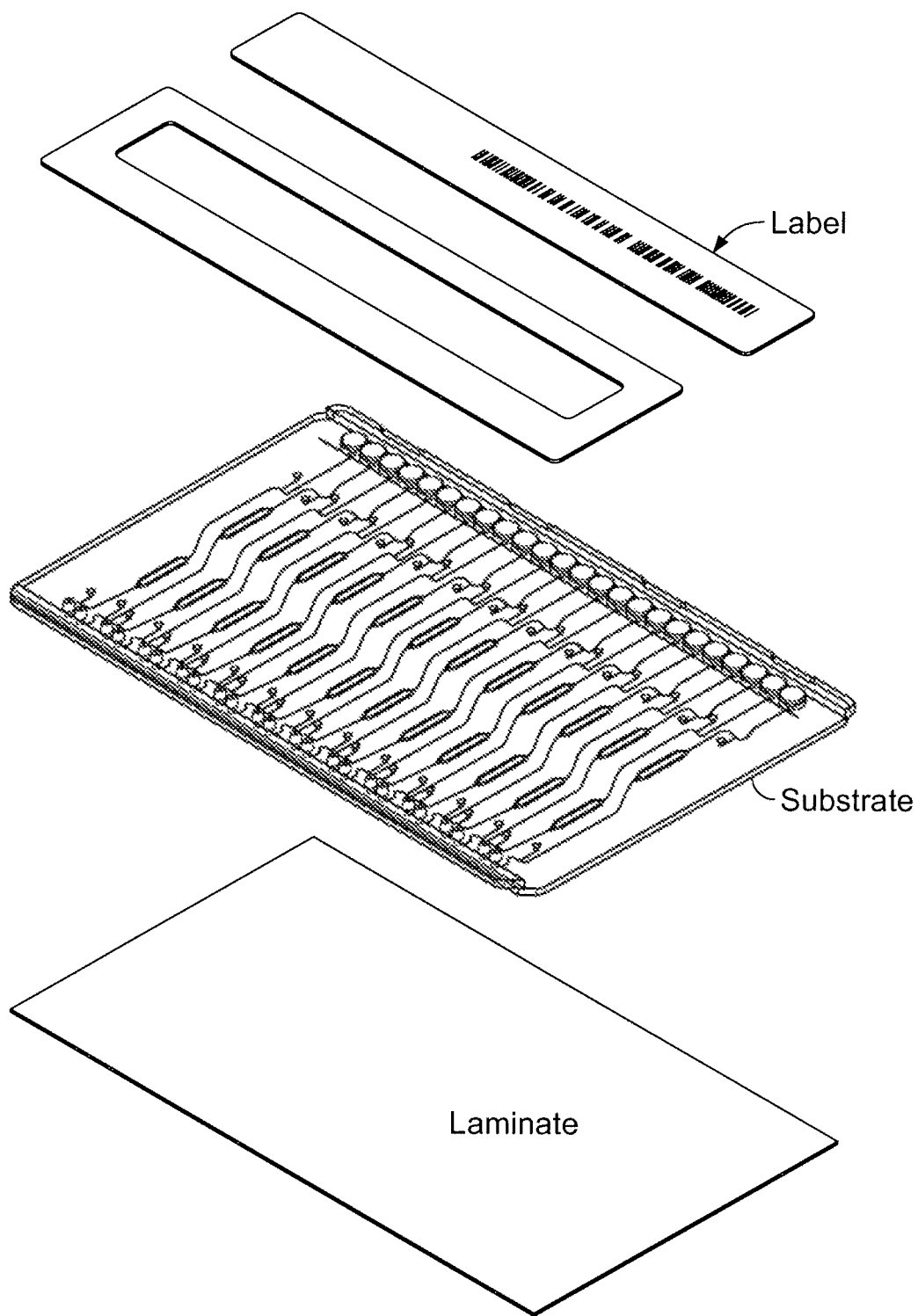
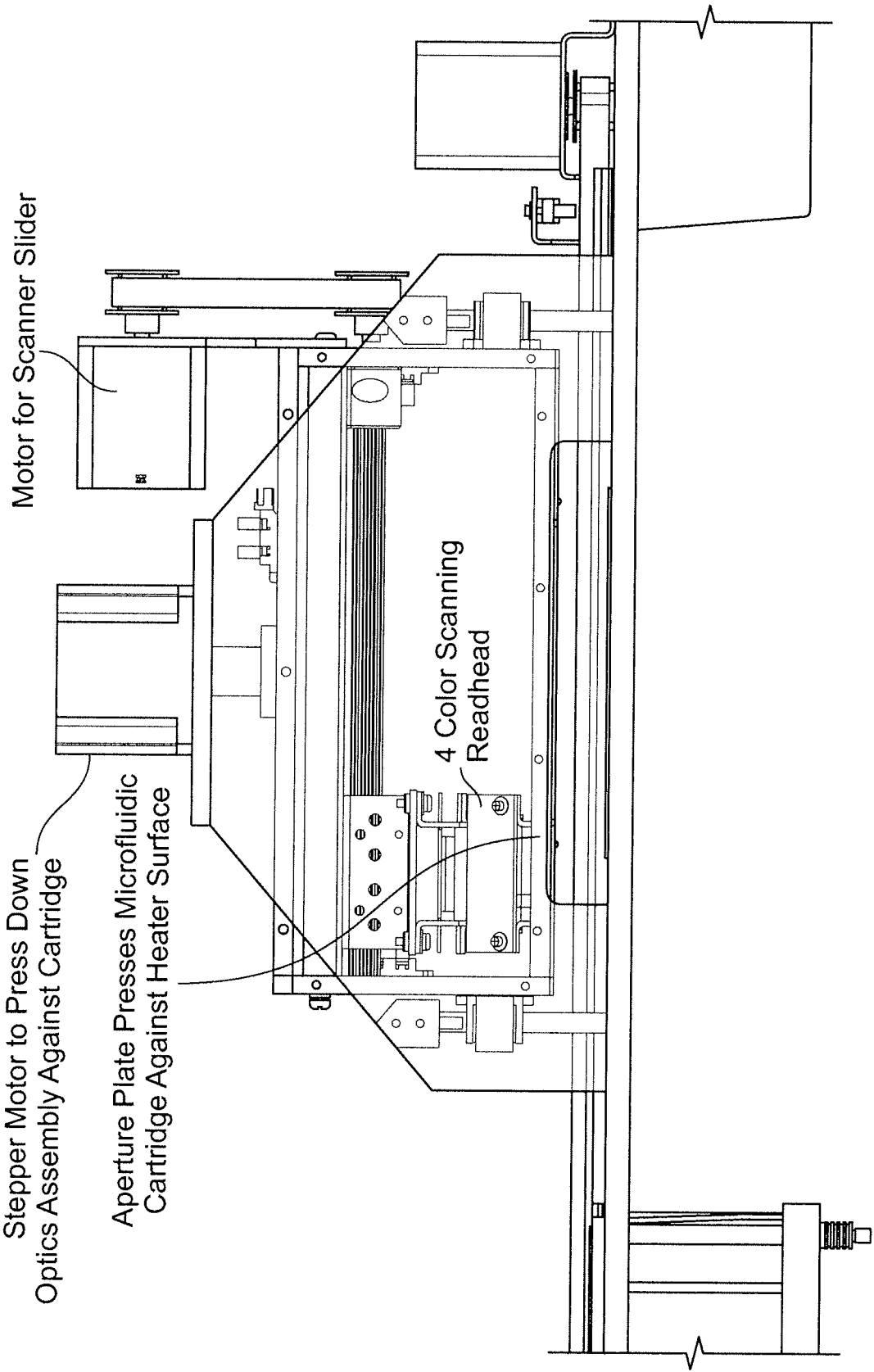


FIG. 77



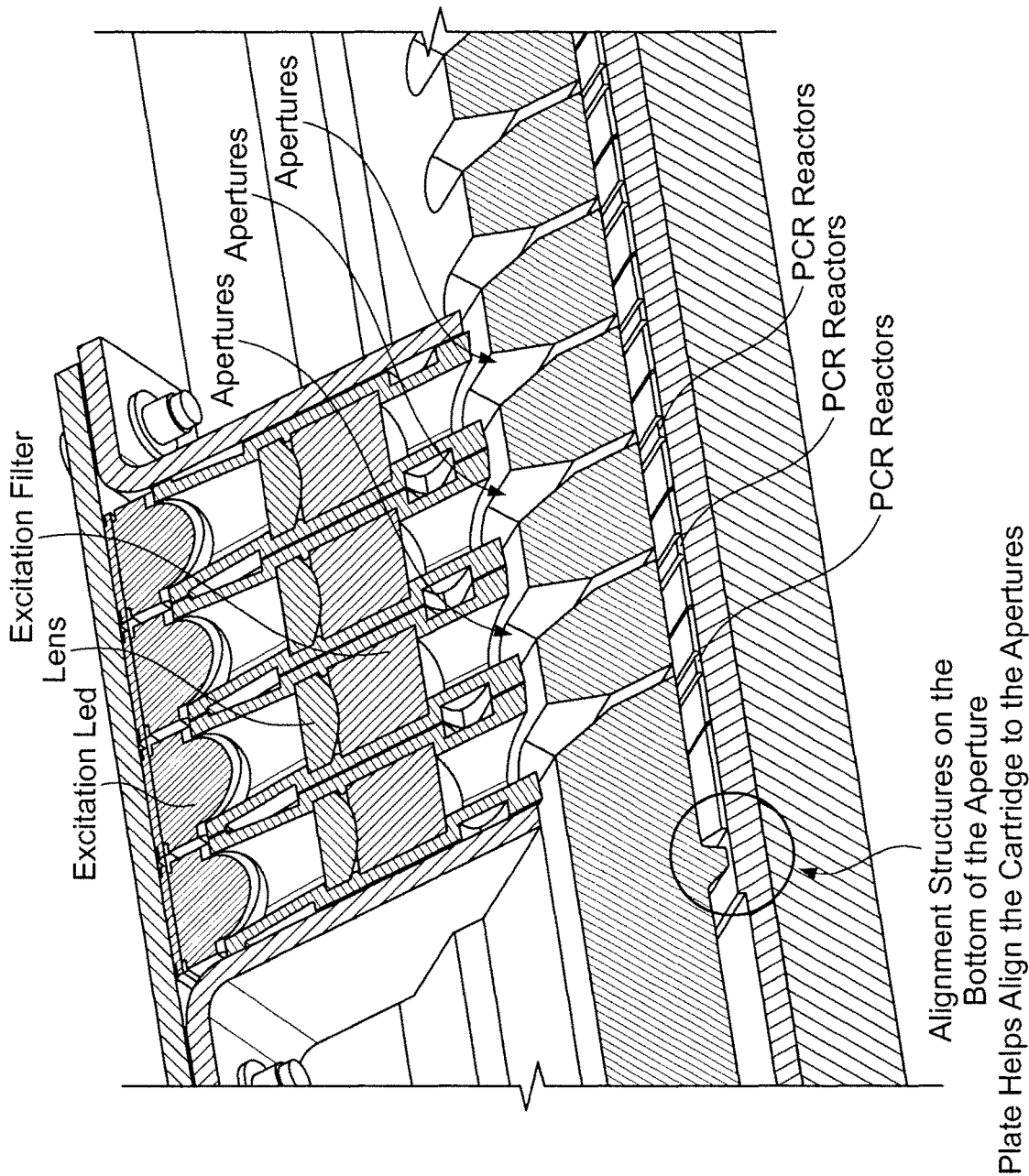


FIG. 79A

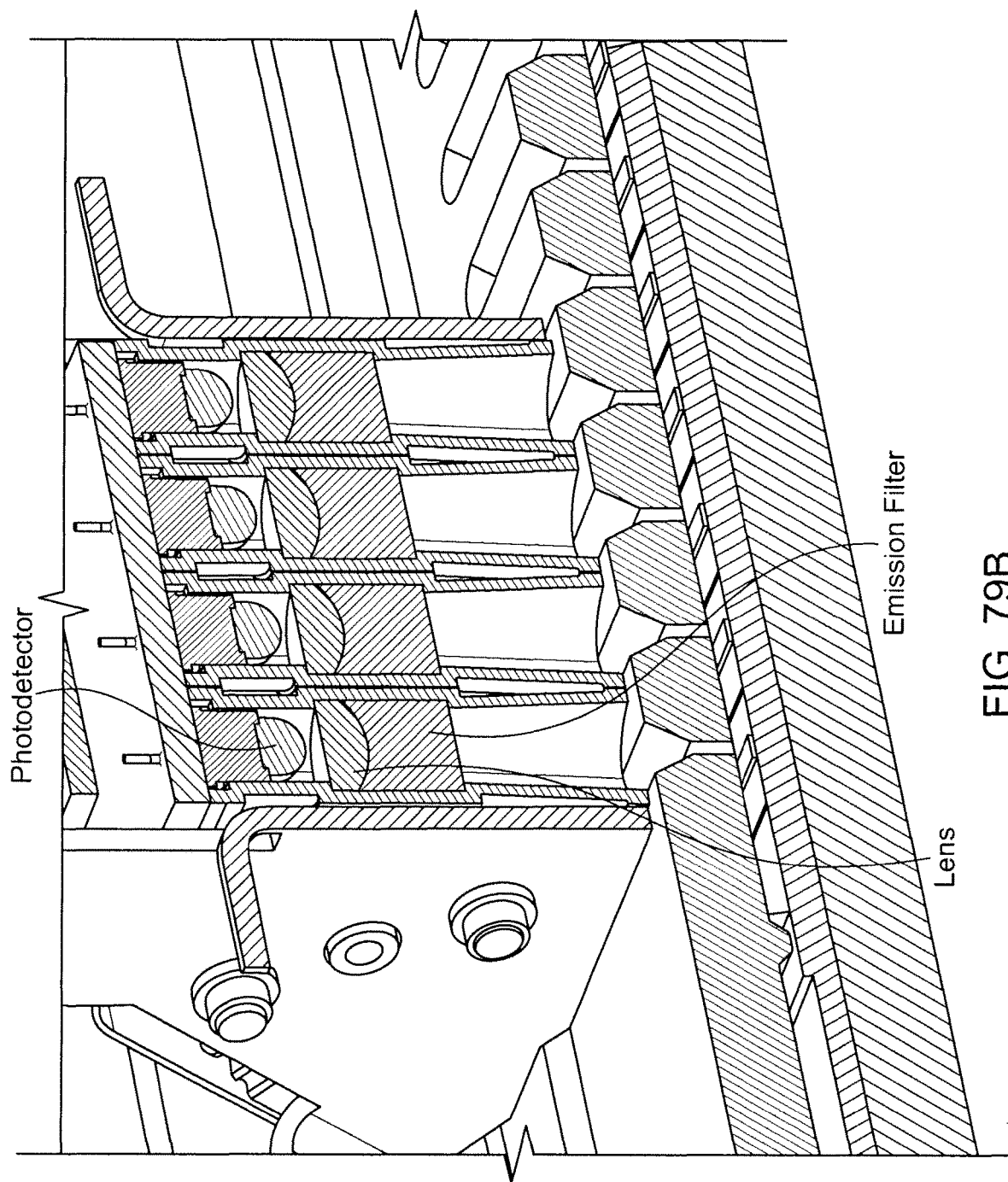
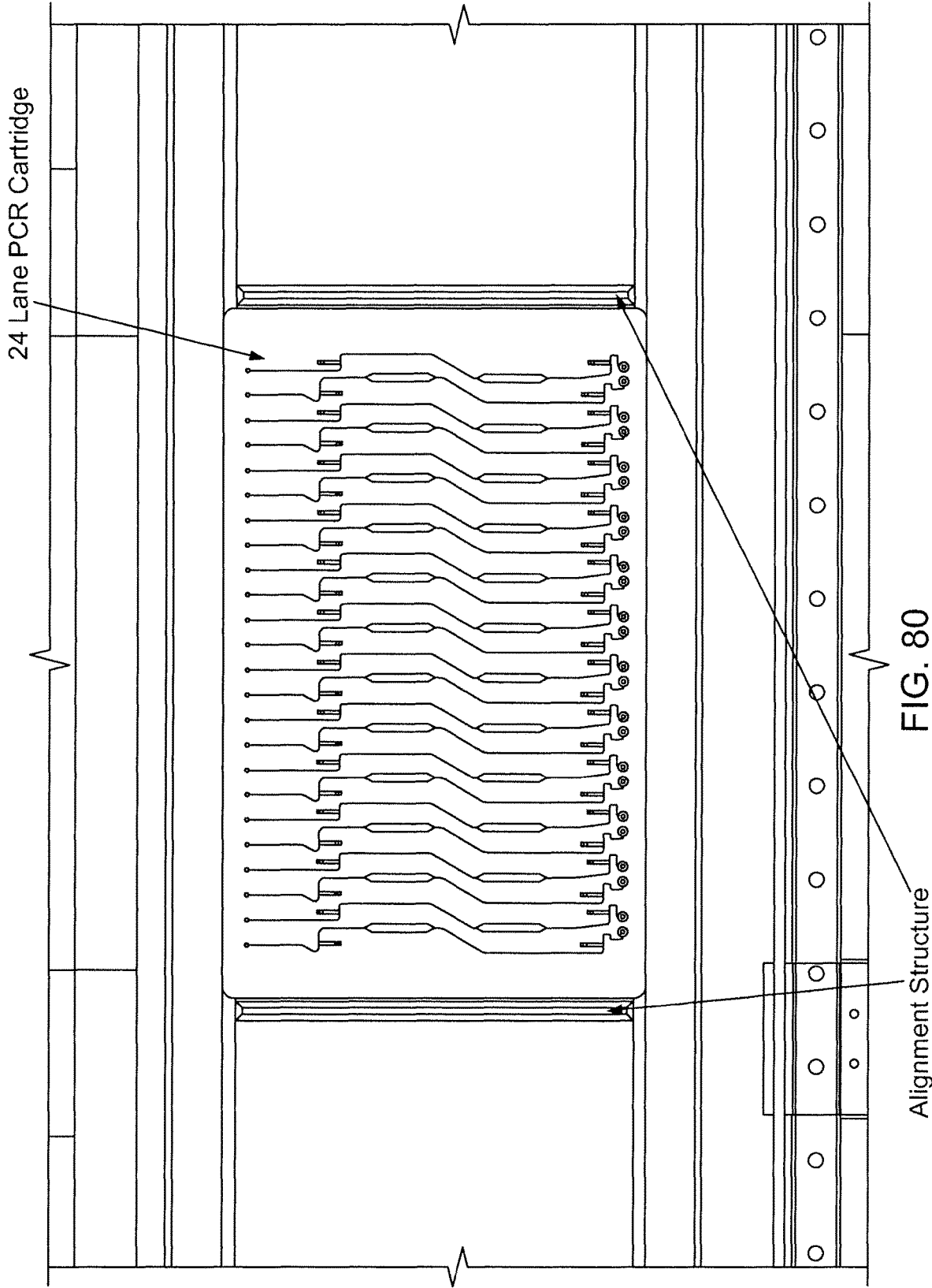
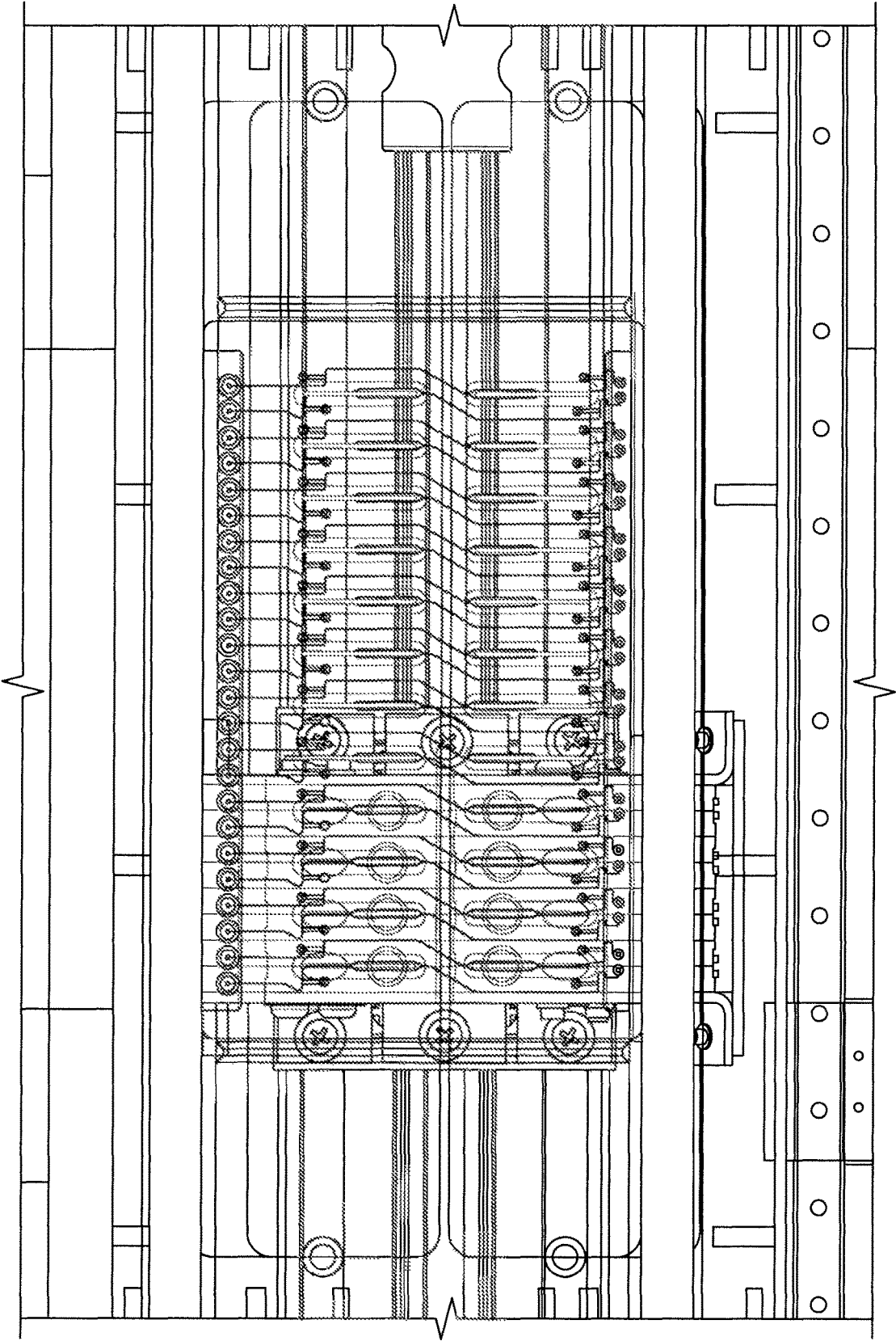


FIG. 79B





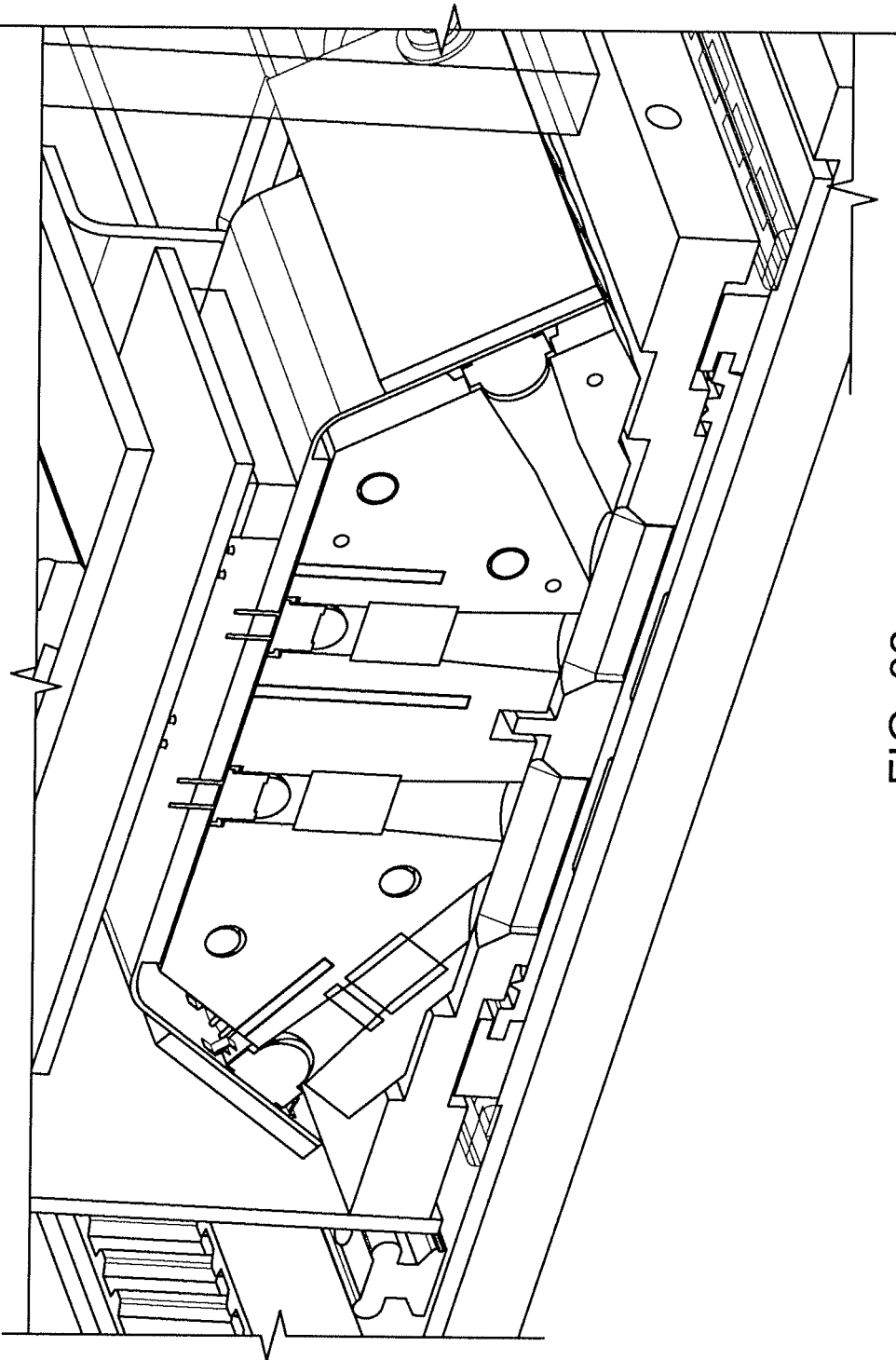


FIG. 82

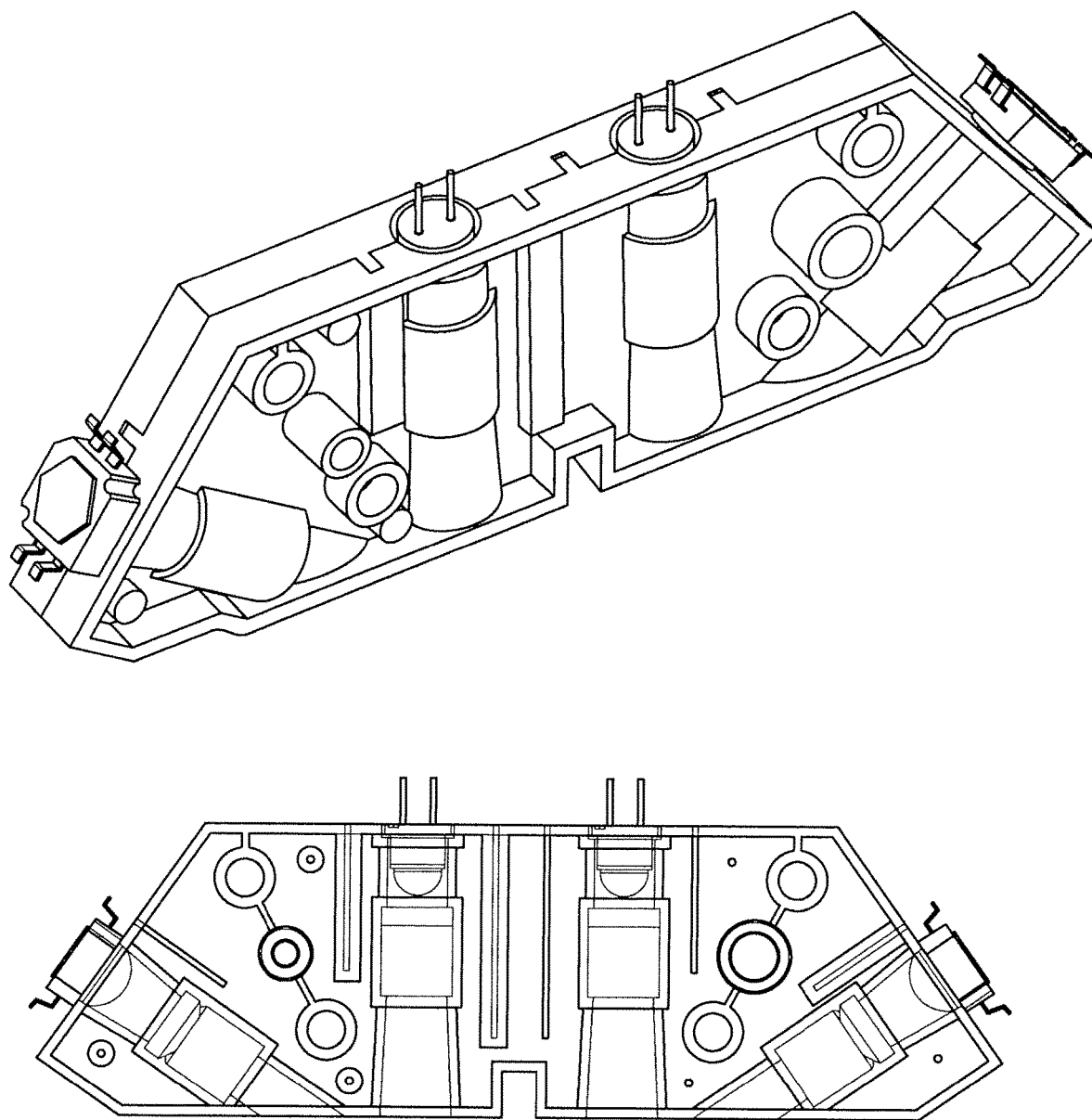


FIG. 83

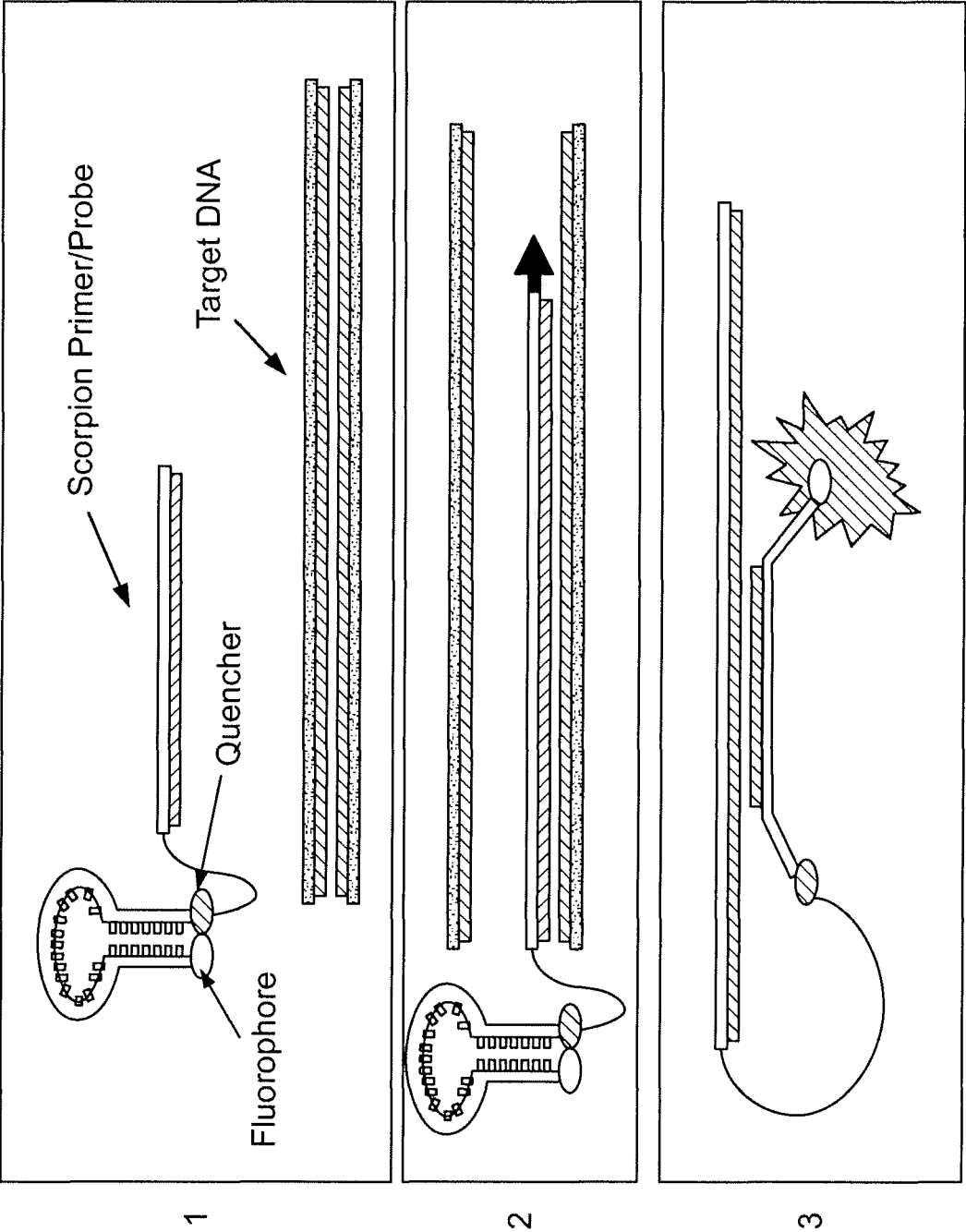


FIG. 84

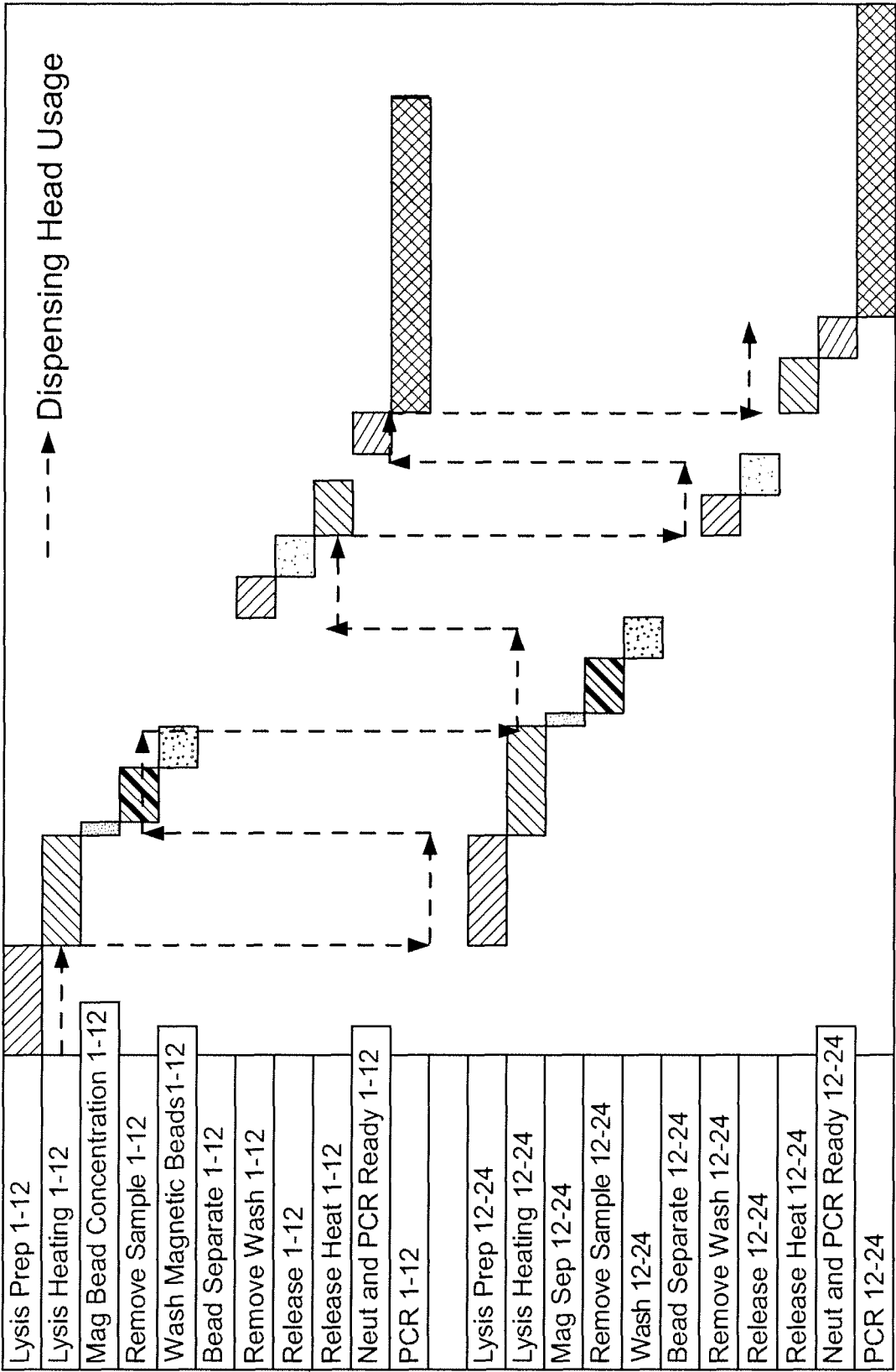


FIG. 85A

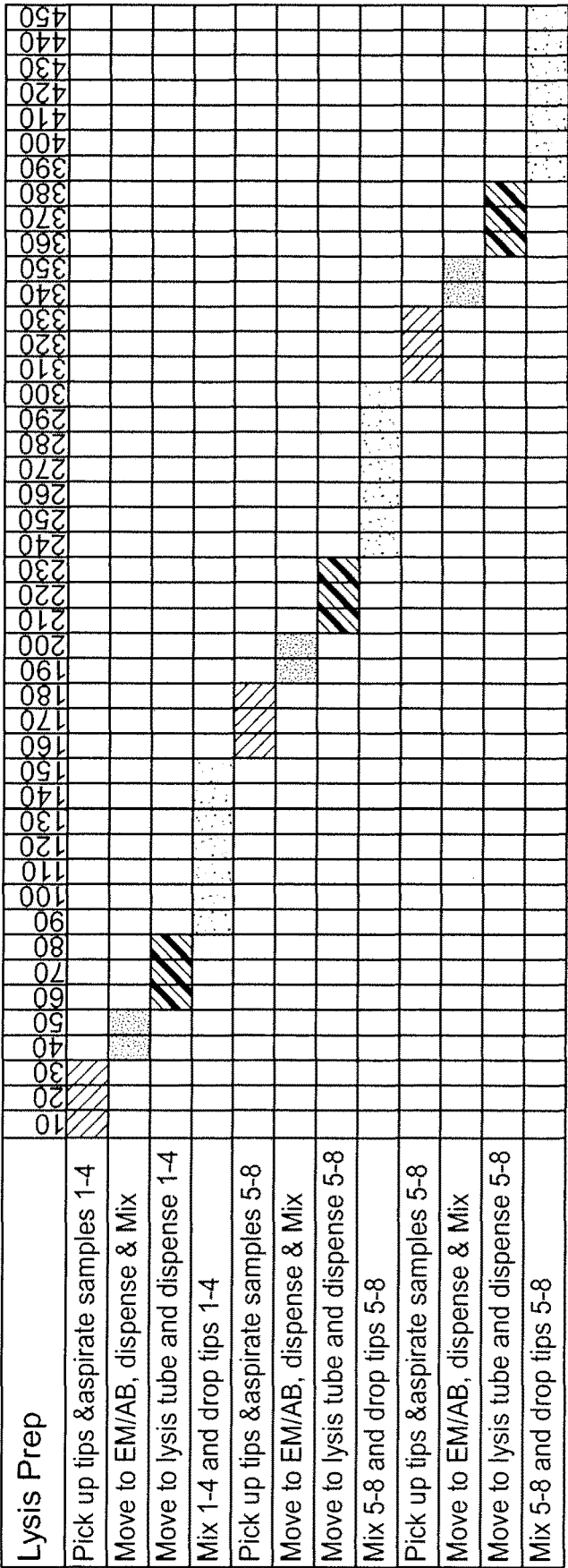


FIG. 85B

[illegible]

FIG. 85C

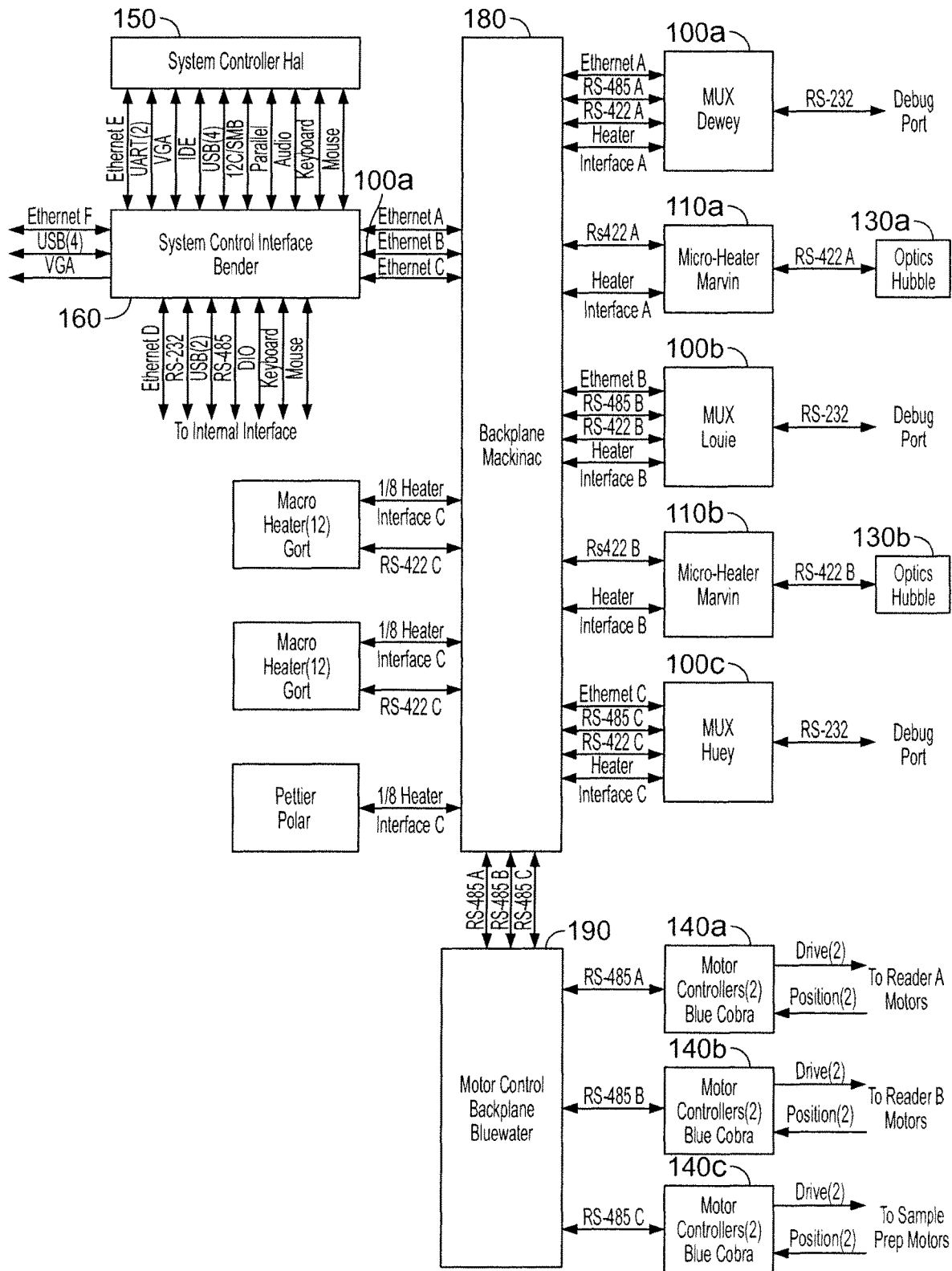


FIG. 86 Electronics Block Diagram

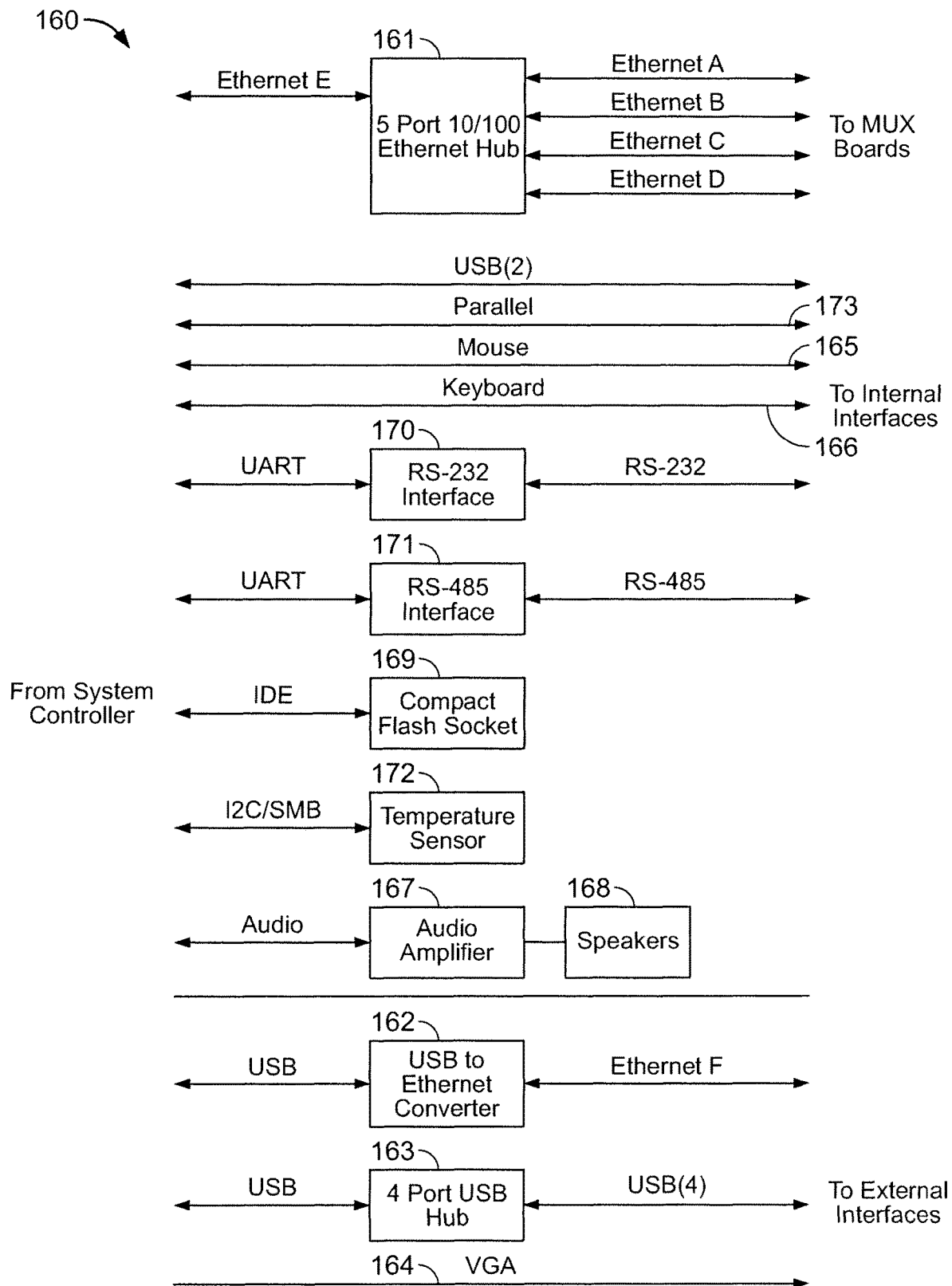


FIG. 87 Processor Base Board Block Diagram

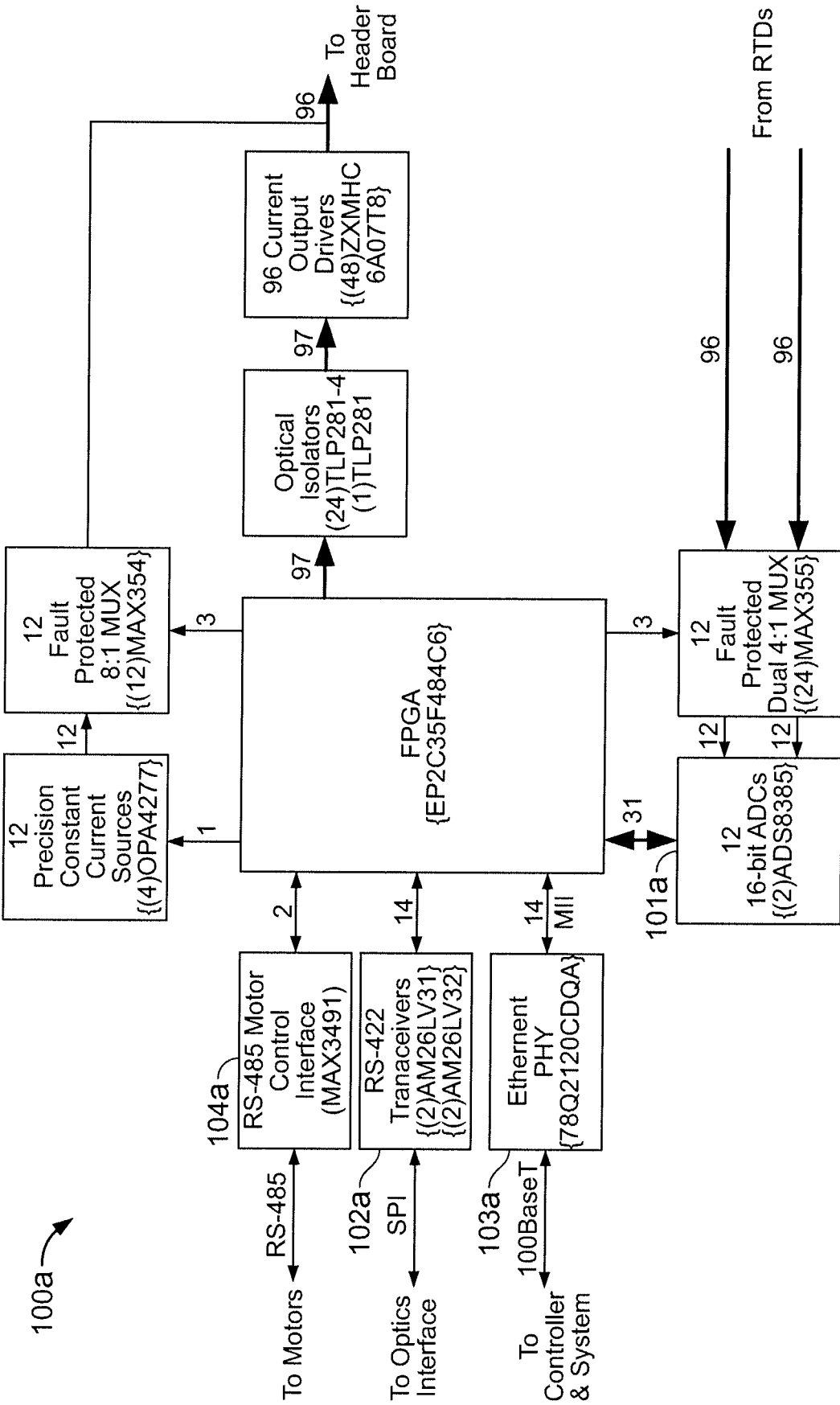


FIG. 88 MUX Board Block Diagram

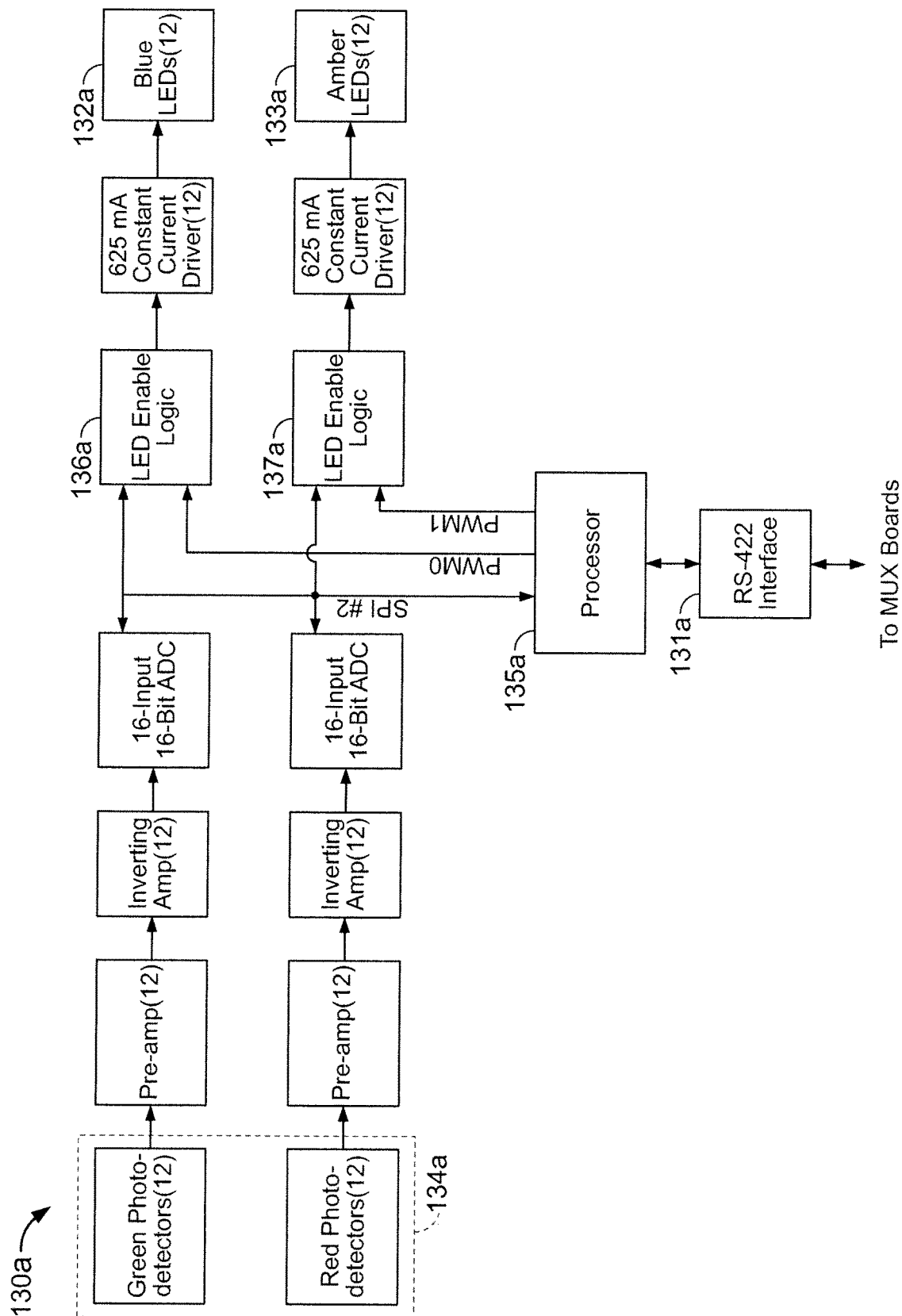


FIG. 89 MUX Board Block Diagram

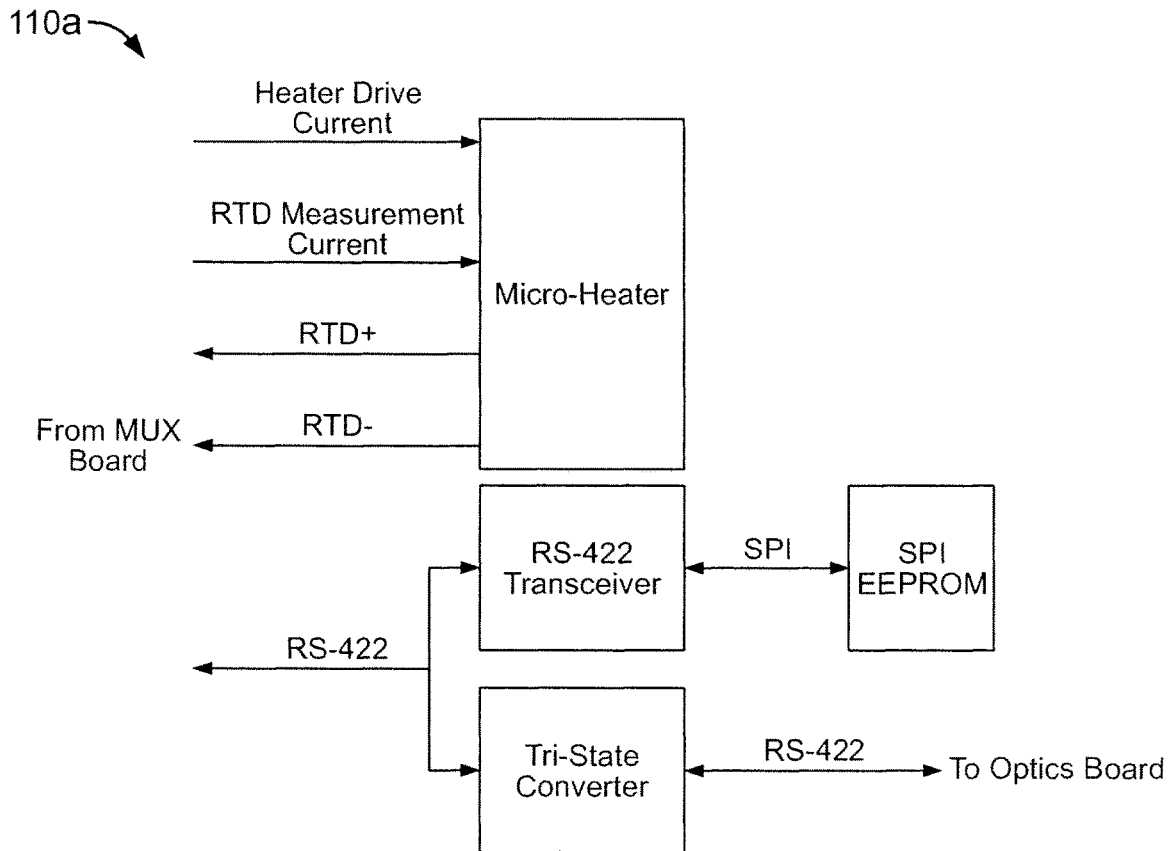


FIG. 90 Micro-Heater Board Block Diagram

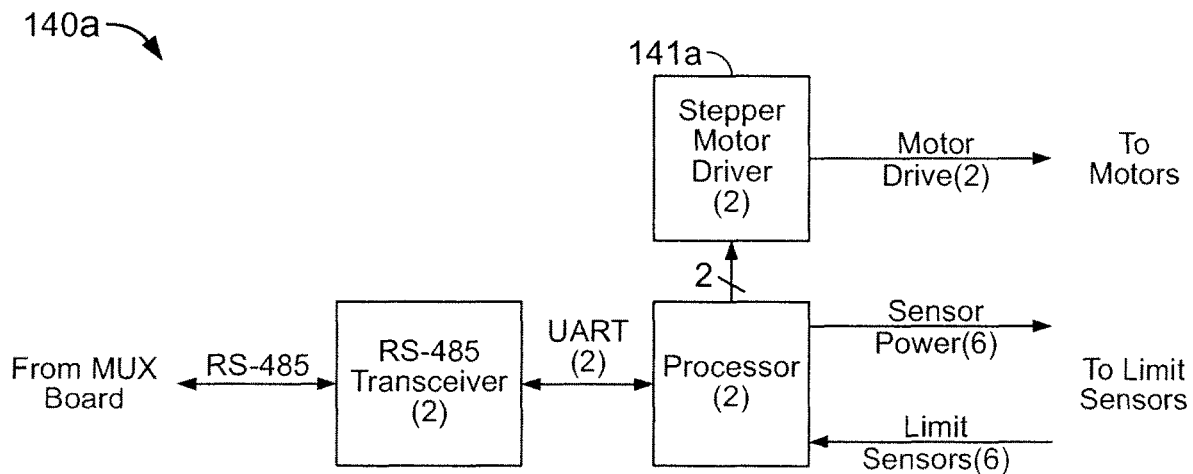


FIG. 91 Motor Control Board Block Diagram

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INTEGRATED APPARATUS FOR PERFORMING NUCLEIC ACID EXTRACTION AND DIAGNOSTIC TESTING ON MULTIPLE BIOLOGICAL SAMPLES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 16/124,672, filed Sep. 7, 2018, which is a continuation of U.S. patent application Ser. No. 14/941,087, filed Nov. 13, 2015 and issued as U.S. Pat. No. 10,071,376 on Sep. 11, 2018, which is a continuation of U.S. patent application Ser. No. 12/218,498, filed Jul. 14, 2008 and issued as U.S. Pat. No. 9,186,677 on Nov. 17, 2015, which claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/959,437, filed Jul. 13, 2007, and is a continuation-in-part of U.S. patent application Ser. No. 11/985,577, filed Nov. 14, 2007 and issued on Aug. 16, 2011 as U.S. Pat. No. 7,998,708. The disclosures of all of the above-referenced prior applications, publications, and patents are considered part of the disclosure of this application, and are incorporated by reference herein in their entirety.

TECHNICAL FIELD

The technology described herein generally relates to systems for extracting polynucleotides from multiple samples, particularly from biological samples, and additionally to systems that subsequently amplify and detect the extracted polynucleotides. The technology more particularly relates to microfluidic systems that carry out PCR on multiple samples of nucleotides of interest within microfluidic channels, and detect those nucleotides.

BACKGROUND

The medical diagnostics industry is a critical element of today's healthcare infrastructure. At present, however, in vitro diagnostic analyses no matter how routine have become a bottleneck in patient care. There are several reasons for this. First, many diagnostic analyses can only be done with highly specialist equipment that is both expensive and only operable by trained clinicians. Such equipment is found in only a few locations—often just one in any given urban area. This means that most hospitals are required to send out samples for analyses to these locations, thereby incurring shipping costs and transportation delays, and possibly even sample loss or mishandling. Second, the equipment in question is typically not available 'on-demand' but instead runs in batches, thereby delaying the processing time for many samples because they must wait for a machine to fill up before they can be run.

Understanding that sample flow breaks down into several key steps, it would be desirable to consider ways to automate as many of these as possible. For example, a biological sample, once extracted from a patient, must be put in a form suitable for a processing regime that typically involves using PCR to amplify a vector (such as a nucleotide) of interest. Once amplified, the presence of a nucleotide of interest from the sample needs to be determined unambiguously. Preparing samples for PCR is currently a time-consuming and labor intensive step, though not one requiring specialist skills, and could usefully be automated. By contrast, steps such as PCR and nucleotide detection (or 'nucleic acid

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testing') have customarily only been within the compass of specially trained individuals having access to specialist equipment.

There is a need for a method and apparatus of carrying out sample preparation on samples in parallel, with or without PCR and detection on the prepared biological samples, and preferably with high throughput, but in a manner that can be done routinely at the point of care, or without needing the sample to be sent out to a specialized facility.

The discussion of the background herein is included to explain the context of the inventions described herein. This is not to be taken as an admission that any of the material referred to was published, known, or part of the common general knowledge as at the priority date of any of the claims.

Throughout the description and claims of the specification the word "comprise" and variations thereof, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

SUMMARY

A diagnostic apparatus, comprising: a first module configured to extract nucleic acid simultaneously from a plurality of nucleic-acid containing samples, wherein the first module comprises: one or more racks, each configured to accept a number of samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain respectively sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample; a magnetic separator configured to move relative to the process chambers of each holder; a heater assembly configured to independently heat each of the process chambers; and a liquid dispenser configured to carry out fluid transfer operations on two or more holders simultaneously; and a second module configured to simultaneously amplify the nucleic acid extracted from the plurality of samples, wherein the second module comprises: one or more bays, each configured to receive a microfluidic cartridge, wherein the cartridge is configured to separately accept and to separately amplify the nucleic acid extracted from multiple samples; and one or more detection systems.

A diagnostic apparatus comprising: one or more racks, on each of which is mounted a number of nucleic acid containing samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain, respectively, sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample; a magnetic separator movable from a first position to a second position adjacent to the process chamber of each of the one or more holders; a heater assembly comprising a number of heater units, each of which is in thermal contact with one of the process chambers; one or more bays, each bay having a shape complementary to a shape of a micro fluidic cartridge, wherein the cartridge comprises a number of inlets each of which is in fluid communication with one of a number of channels in which nucleic acid extracted from one of the number of samples is amplified, and wherein the cartridge further comprises one or more windows that permit detection of amplified nucleic acid; a liquid dispenser having one or more dispensing heads, wherein the liquid dispenser is movable from a first position above a first holder to a second position above a second holder, and is movable from the first

position above the first holder to a different position above the first holder, and is further movable from a position above one of the holders to a position above one of the number of inlets; and one or more detection systems positioned in proximity to the one or more windows.

A diagnostic instrument comprising: a liquid handling unit that extracts nucleic acid from a sample in a unitized reagent strip; a microfluidic cartridge that, in conjunction with a heater element, carries out real-time PCR on nucleic acid extracted from the sample; and a detector that provides a user with a diagnosis of whether the sample contains a nucleotide of interest.

Also described herein are methods of using the diagnostic apparatus, including a method of diagnosing a number of samples in parallel, using the apparatus.

A unitized reagent holder, comprising: a strip, to which is attached: a single process tube; one or more receptacles, each of which holding a reagent selected from the group consisting of: a sample preparation reagent, PCR reagents for a first analyte, and one or more liquid reagents; a waste tube; one or more sockets configured to hold one or more pipette tips; and a pipette tip sheath configured to surround the one or more pipette tips.

A liquid dispenser, comprising: one or more sensors; a manifold; one or more pumps in fluid communication with the manifold; one or more dispense heads in fluid communication with the manifold; a gantry that provides freedom of translational motion in three dimensions; and electrical connections that accept electrical signals from an external controller, wherein the liquid dispenser has no inlet or outlet for fluids, other than through the one or more pumps.

A separator for magnetic particles, comprising: one or more magnets aligned linearly; a motorized shaft upon which the one or more magnets can rise or fall in such a manner that the one or more magnets attains close proximity to one or more receptacles containing magnetic particles; and control circuitry to control motion of the motorized shaft.

An integrated separator and heater, comprising: a heater assembly, wherein the heater assembly comprises a plurality of independently controllable heater units, each of which is configured to accept and to heat a process chamber; one or more magnets aligned linearly; a motorized shaft upon which the one or more magnets can rise or fall in such a manner that the one or more magnets attains close proximity to one or more of the process chambers; and control circuitry to control motion of the motorized shaft and heating of the heater units.

A preparatory apparatus comprising: a first module configured to extract nucleic acid simultaneously from a number of nucleic-acid containing samples, wherein the first module comprises: one or more racks, each configured to accept the number of samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain, respectively, sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample; a magnetic separator configured to move relative to the process chambers of each holder; a heater assembly configured to independently heat each of the process chambers; and a liquid dispenser configured to carry out fluid transfer operations on two or more holders simultaneously; and a second module configured to receive and to store the nucleic acid extracted from the number of samples.

A preparatory apparatus comprising: one or more racks, on each of which is mounted a number of nucleic acid

containing samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain, respectively, sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample; a magnetic separator movable from a first position to a second position adjacent to the process chambers of each holder; a heater assembly comprising a number of heater units, each of which is in contact with a process chamber; a liquid dispenser movable from a first position above a first holder to a second position above a second holder; and a storage compartment having a number of compartments, wherein each compartment stores the nucleic acid extracted from one of the number of samples.

A unitized reagent holder, comprising: a strip, to which is attached: a single process tube; one or more receptacles, each of which holding a reagent selected from the group consisting of: a sample preparation reagent, and one or more liquid reagents; a waste tube; one or more sockets configured to hold one or more pipette tips; and a pipette tip sheath configured to surround the one or more pipette tips.

The present technology additionally includes a process for extracting nucleic acid from multiple samples in parallel, using the apparatus as described herein.

BRIEF DESCRIPTION OF SELECTED DRAWINGS

FIG. 1A shows a schematic of a preparatory apparatus; FIG. 1B shows a schematic of a diagnostic apparatus.

FIG. 2 shows a schematic of control circuitry.

FIGS. 3A and 3B show exterior views of an exemplary apparatus.

FIG. 4 shows an exemplary interior view of an apparatus.

FIG. 5 shows perspective views of an exemplary rack for sample holders.

FIG. 6 shows perspective views of the rack of FIG. 5 in conjunction with a heater unit.

FIG. 7 shows a perspective view of an exemplary rack for sample holders.

FIGS. 8A-8K show various views of the rack of FIG. 7.

FIG. 9 shows an area of an apparatus configured to accept a rack of FIG. 7.

FIGS. 10A and 10B show a first exemplary embodiment of a reagent holder having a pipette sheath, in perspective view (FIG. 10A) and underside view (FIG. 10B).

FIG. 11 shows an exemplary embodiment of a reagent holder not having a pipette sheath, in perspective view.

FIGS. 12A-12C show a second exemplary embodiment of a reagent holder having a pipette sheath, in perspective view (FIG. 12A) and cross-sectional view (FIG. 12B), and exploded view (FIG. 12C).

FIGS. 13A and 13B show a stellated feature on the interior of a reagent tube, in cross-sectional (FIG. 13A) and plan (FIG. 13B) view.

FIG. 14 shows a sequence of pipetting operations in conjunction with a reagent tube having a stellated feature.

FIG. 15 shows embodiments of a laminated layer.

FIG. 16 shows a sequence of pipetting operations in conjunction with a laminated layer.

FIGS. 17A-17D show an exemplary kit containing holders and reagents.

FIG. 18 shows a liquid dispense head.

FIGS. 19A-19C show a liquid dispense head.

FIG. 20 shows an exemplary distribution manifold.

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FIG. 21 shows a scanning read-head attached to a liquid dispense head.

FIG. 22 shows a barcode scanner in cross-sectional view.

FIG. 23 shows a barcode reader positioned above a microfluidic cartridge.

FIG. 24 shows pipette tip sensors.

FIGS. 25A and 25B show an exemplary device for stripping pipette tip.

FIG. 26 shows a heater unit in perspective and cross-sectional view.

FIG. 27 shows an integrated heater and separator unit in cross-sectional view.

FIG. 28 shows a cartridge auto-loader.

FIG. 29 shows a cartridge stacker.

FIG. 30 shows a cartridge stacker in position to deliver a cartridge to an auto-loader.

FIG. 31 shows a cartridge loading system.

FIG. 32 shows a disposal unit for used cartridges.

FIG. 33 shows a cartridge stacker in full and empty configurations.

FIG. 34 shows a microfluidic cartridge, a read-head, and a cartridge tray.

FIG. 35 shows a cross-section of a pipetting head and a cartridge in position in a microfluidic apparatus.

FIG. 36 shows an exemplary microfluidic cartridge having a 3-layer construction.

FIG. 37 shows a plan of microfluidic circuitry and inlets in an exemplary multi-lane cartridge.

FIG. 38A shows an exemplary multi-lane cartridge.

FIG. 38B shows a portion of an exemplary multi-lane cartridge.

FIGS. 39A, 39B show an exemplary microfluidic network in a lane of a multi-lane cartridge;

FIGS. 40A-40C show diagrams of exemplary microfluidic valves. FIG. 40A additionally shows the valve in an open state, and the valve in a closed state.

FIG. 41 shows a vent.

FIG. 42 shows an exemplary highly-multiplexed microfluidic cartridge;

FIGS. 43-46 show various aspects of exemplary highly multiplexed microfluidic cartridges; and

FIGS. 47A-C show various aspects of a radially configured highly multiplexed microfluidic cartridge.

FIG. 48 shows a view in cross-section of a microfluidic cartridge.

FIGS. 49A, 49B show a PCR reaction chamber and associated heaters.

FIG. 50 shows thermal images of heater circuitry in operation.

FIGS. 51A-51C shows various cut-away sections that can be used to improve cooling rates during PCR thermal cycling.

FIG. 52 shows a plot of temperature against time during a PCR process, as performed on a microfluidic cartridge as described herein.

FIG. 53 shows an assembly process for a cartridge as further described herein.

FIGS. 54A and 54B show exemplary apparatus for carrying out wax deposition.

FIGS. 55A and 55B show exemplary deposition of wax droplets into microfluidic valves.

FIG. 56 shows an overlay of an array of heater elements on an exemplary multi-lane microfluidic cartridge, wherein various microfluidic networks are visible.

FIG. 57 shows a cross-sectional view of an exemplary detector.

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FIG. 58 shows a perspective view of a detector in a read-head.

FIG. 59 shows a cutaway view of an exemplary detector in a read-head.

FIG. 60 shows an exterior view of an exemplary multiplexed read-head with an array of detectors therein.

FIG. 61 shows an cutaway view of an exemplary multiplexed read-head with an array of detectors therein.

FIG. 62 shows a block diagram of exemplary electronic circuitry in conjunction with a detector as described herein.

FIG. 63 shows an exemplary liquid dispensing system.

FIG. 64 shows an exemplary heater/separator.

FIGS. 65A and 65B show exemplary aspects of a computer-based user interface.

FIG. 66 shows schematically layout of components of a preparatory apparatus.

FIG. 67 shows layout of components of an exemplary preparatory apparatus.

FIG. 68 shows schematically layout of components of a diagnostic apparatus.

FIG. 69 shows layout of components of an exemplary diagnostic apparatus.

FIGS. 70 and 71 show exterior and interior of an exemplary diagnostic apparatus.

FIGS. 72A and 72B show a thermocycling unit configured to accept a microfluidic cartridge.

FIG. 73 shows schematically a layout of components of a high-efficiency diagnostic apparatus.

FIG. 74 shows layout of components of an exemplary high-efficiency diagnostic apparatus.

FIG. 75 shows a plan view of a 24-lane microfluidic cartridge.

FIG. 76 shows a perspective view of the cartridge of FIG. 75.

FIG. 77 shows an exploded view of the cartridge of FIG. 75.

FIG. 78 shows an exemplary detection unit.

FIGS. 79A, 79B show cutaway portions of the detection unit of FIG. 78.

FIGS. 80, and 81 show alignment of the detection unit with a microfluidic cartridge.

FIGS. 82 and 83 show exterior and cutaways, respectively, of an optics block.

FIG. 84 shows a Scorpion reaction, schematically.

FIGS. 85A-85C show, schematically, pipette head usage during various preparatory processes.

FIGS. 86-91 show exemplary layouts of electronics control circuitry.

DETAILED DESCRIPTION

Nucleic acid testing (NAT) as used herein is a general term that encompasses both DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid) testing. Exemplary protocols that are specific to RNA and to DNA are described herein. It is to be understood that generalized descriptions where not specific to RNA or to DNA either apply to each equally or can be readily adapted to either with minor variations of the description herein as amenable to one of ordinary skill in the art. It is also to be understood that the terms nucleic acid and polynucleotide may be used interchangeably herein.

The apparatuses as described herein therefore find application to analyzing any nucleic acid containing sample for any purpose, including but not limited to genetic testing, and clinical testing for various infectious diseases in humans. Targets for which clinical assays currently exist, and that may be tested for using the apparatus and methods herein

may be bacterial or viral, and include, but are not limited to: *Chlamydia trachomatis* (CT); *Neisseria gonorrhea* (GC); Group B *Streptococcus*; HSV; HSV Typing; CMV; Influenza A & B; MRSA; RSV; TB; *Trichomonas*; Adenovirus; Bordetella; BK; JC; HHV6; EBV; Enterovirus; and *M. pneumoniae*.

The apparatus herein can be configured to run on a laboratory benchtop, or similar environment, and can test approximately 45 samples per hour when run continuously throughout a normal working day. This number can be increased, according to the number of tests that can be accommodated in a single batch, as will become clear from the description herein. Results from individual raw samples are typically available in less than 1 hour.

Where used herein, the term “module” should be taken to mean an assembly of components, each of which may have separate, distinct and/or independent functions, but which are configured to operate together to produce a desired result or results. It is not required that every component within a module be directly connected or in direct communication with every other. Furthermore, connectivity amongst the various components may be achieved with the aid of a component, such as a processor, that is external to the module.

Apparatus Overview

An apparatus having various components as further described herein can be configured into at least two formats, preparatory and diagnostic, as shown respectively in FIGS. 1A and 1B. A schematic overview of a preparatory apparatus 981 for carrying out sample preparation as further described herein is shown in FIG. 1A. An overview of a diagnostic apparatus 971 is shown in FIG. 1B. The geometric arrangement of the components of systems 971, 981 shown in FIGS. 1A and 1B is exemplary and not intended to be limiting.

A processor 980, such as a microprocessor, is configured to control functions of various components of the system as shown, and is thereby in communication with each such component requiring control. It is to be understood that many such control functions can optionally be carried out manually, and not under control of the processor. Furthermore, the order in which the various functions are described, in the following, is not limiting upon the order in which the processor executes instructions when the apparatus is operating. Thus, processor 980 can be configured to receive data about a sample to be analyzed, e.g., from a sample reader 990, which may be a barcode reader, an optical character reader, or an RFID scanner (radio frequency tag reader). It is also to be understood that, although a single processor 980 is shown as controlling all operations of apparatus 971 and 981, such operations may be distributed, as convenient, over more than one processor.

Processor 980 can be configured to accept user instructions from an input 984, where such instructions may include instructions to start analyzing the sample, and choices of operating conditions. Although not shown in FIGS. 1A and 1B, in various embodiments, input 984 can include one or more input devices selected from the group consisting of: a keyboard, a touch-sensitive surface, a microphone, a track-pad, a retinal scanner, a holographic projection of an input device, and a mouse. A suitable input device may further comprise a reader of formatted electronic media, such as, but not limited to, a flash memory card, memory stick, USB-stick, CD, or floppy diskette. An input device may further comprise a security feature such as a fingerprint reader, retinal scanner, magnetic strip reader, or bar-code reader, for ensuring that a user of the system is in fact authorized to do so, according to pre-loaded identifying

characteristics of authorized users. An input device may additionally—and simultaneously—function as an output device for writing data in connection with sample analysis. For example, if an input device is a reader of formatted electronic media, it may also be a writer of such media. Data that may be written to such media by such a device includes, but is not limited to, environmental information, such as temperature or humidity, pertaining to an analysis, as well as a diagnostic result, and identifying data for the sample in question.

Processor 980 can be also configured to communicate with a display 982, so that, for example, information about an analysis is transmitted to the display and thereby communicated to a user of the system. Such information includes but is not limited to: the current status of the apparatus; progress of PCR thermocycling; and a warning message in case of malfunction of either system or cartridge. Additionally, processor 980 may transmit one or more questions to be displayed on display 982 that prompt a user to provide input in response thereto. Thus, in certain embodiments, input 984 and display 982 are integrated with one another.

Processor 980 can be optionally further configured to transmit results of an analysis to an output device such as a printer, a visual display, a display that utilizes a holographic projection, or a speaker, or a combination thereof.

Processor 980 can be still further optionally connected via a communication interface such as a network interface to a computer network 988. The communication interface can be one or more interfaces selected from the group consisting of: a serial connection, a parallel connection, a wireless network connection, a USB connection, and a wired network connection. Thereby, when the system is suitably addressed on the network, a remote user may access the processor and transmit instructions, input data, or retrieve data, such as may be stored in a memory (not shown) associated with the processor, or on some other computer-readable medium that is in communication with the processor. The interface may also thereby permit extraction of data to a remote location, such as a personal computer, personal digital assistant, or network storage device such as computer server or disk farm. The apparatus may further be configured to permit a user to e-mail results of an analysis directly to some other party, such as a healthcare provider, or a diagnostic facility, or a patient.

Additionally, in various embodiments, the apparatus can further comprise a data storage medium configured to receive data from one or more of the processor, an input device, and a communication interface, the data storage medium being one or more media selected from the group consisting of: a hard disk drive, an optical disk drive, a flash card, and a CD-Rom.

Processor 980 can be further configured to control various aspects of sample preparation and diagnosis, as follows in overview, and as further described in detail herein. In FIGS. 1A and 1B, the apparatus 981 (or 971) is configured to operate in conjunction with a complementary rack 970. The rack is itself configured, as further described herein, to receive a number of biological samples 996 in a form suitable for work-up and diagnostic analysis, and a number of holders 972 that are equipped with various reagents, pipette tips and receptacles. The rack is configured so that, during sample work-up, samples are processed in the respective holders, the processing including being subjected, individually, to heating and cooling via heater assembly 977. The heating functions of the heater assembly can be controlled by the processor 980. Heater assembly 977 operates in conjunction with a separator 978, such as a magnetic

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separator, that also can be controlled by processor 980 to move into and out of close proximity to one or more processing chambers associated with the holders 972, wherein particles such as magnetic particles are present.

Liquid dispenser 976, which similarly can be controlled by processor 980, is configured to carry out various suck and dispense operations on respective sample, fluids and reagents in the holders 972, to achieve extraction of nucleic acid from the samples. Liquid dispenser 976 can carry out such operations on multiple holders simultaneously. Sample reader 990 is configured to transmit identifying indicia about the sample, and in some instances the holder, to processor 980. In some embodiments a sample reader is attached to the liquid dispenser and can thereby read indicia about a sample above which the liquid dispenser is situated. In other embodiments the sample reader is not attached to the liquid dispenser and is independently movable, under control of the processor. Liquid dispenser 976 is also configured to take aliquots of fluid containing nucleic acid extracted from one or more samples and direct them to storage area 974, which may be a cooler. Area 974 contains, for example, a PCR tube corresponding to each sample. In other embodiments, there is not a separate Area 974, but a cooler can be configured to cool the one or more holders 972 so that extracted nucleic acid is cooled and stored in situ rather than being transferred to a separate location.

FIG. 1B shows a schematic embodiment of a diagnostic apparatus 971, having elements in common with apparatus 981 FIG. 1A but, in place of a storage area 974, having a receiving bay 992 in which a cartridge 994 is received. The receiving bay is in communication with a heater 998 that itself can be controlled by processor 980 in such a way that specific regions of the cartridge are heated at specific times during analysis. Liquid dispenser 976 is thus configured to take aliquots of fluid containing nucleic acid extracted from one or more samples and direct them to respective inlets in cartridge 994. Cartridge 994 is configured to amplify, such as by carrying out PCR, on the respective nucleic acids. The processor is also configured to control a detector 999 that receives an indication of a diagnosis from the cartridge 994. The diagnosis can be transmitted to the output device 986 and/or the display 982, as described hereinabove.

A suitable processor 980 can be designed and manufactured according to, respectively, design principles and semiconductor processing methods known in the art.

Embodiments of the apparatuses shown in outline in FIGS. 1A and 1B, as with other exemplary embodiments described herein, is advantageous because they do not require locations within the apparatus suitably configured for storage of reagents. Neither do embodiments of the system, or other exemplary embodiments herein, require inlet or outlet ports that are configured to receive reagents from, e.g., externally stored containers such as bottles, canisters, or reservoirs. Therefore, the apparatuses in FIGS. 1A and 1B are self-contained and operate in conjunction with holders 972, wherein the holders are pre-packaged with reagents, such as in locations within it dedicated to reagent storage.

The apparatuses of FIGS. 1A and 1B may be configured to carry out operation in a single location, such as a laboratory setting, or may be portable so that they can accompany, e.g., a physician, or other healthcare professional, who may visit patients at different locations. The apparatuses are typically provided with a power-cord so that they can accept AC power from a mains supply or generator. An optional transformer (not shown) built into each apparatus, or situated externally between a power socket and the

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system, transforms AC input power into a DC output for use by the apparatus. The apparatus may also be configured to operate by using one or more batteries and therefore is also typically equipped with a battery recharging system, and various warning devices that alert a user if battery power is becoming too low to reliably initiate or complete a diagnostic analysis.

The apparatuses of FIGS. 1A and 1B may further be configured, in other embodiments, for multiplexed sample analysis and/or analysis of multiple batches of samples, where, e.g., a single rack holds a single batch of samples. In one such configuration, instances of a system, as outlined in FIG. 1B, accept and to process multiple microfluidic cartridges 994. Each component shown in FIGS. 1A and 1B may therefore be present as many times as there are batches of samples, though the various components may be configured in a common housing.

In still another configuration, a system is configured to accept and to process multiple cartridges, but one or more components in FIGS. 1A and 1B is common to multiple cartridges. For example, a single apparatus may be configured with multiple cartridge receiving bays, but a common processor, detector, and user interface suitably configured to permit concurrent, consecutive, or simultaneous, control of the various cartridges. It is further possible that such an embodiment, also utilizes a single sample reader, and a single output device.

In still another configuration, a system as shown in FIG. 1B is configured to accept a single cartridge, wherein the single cartridge is configured to process more than 1, for example, 2, 3, 4, 5, or 6, samples in parallel, and independently of one another. Exemplary technology for creating cartridges that can handle multiple samples is described elsewhere, e.g., in U.S. application Ser. No. 60/859,284, incorporated herein by reference.

It is further consistent with the present technology that a cartridge can be tagged, e.g., with a molecular bar-code indicative of the sample, to facilitate sample tracking, and to minimize risk of sample mix-up. Methods for such tagging are described elsewhere, e.g., in U.S. patent application Ser. No. 10/360,854, incorporated herein by reference.

Control electronics 840 implemented into apparatus 971 or 981, shown schematically in the block diagram in FIG. 2, can include one or more functions in various embodiments, for example, for main control 900, multiplexing 902, display control 904, detector control 906, and the like. The main control function may serve as the hub of control electronics 840 in the apparatuses of FIGS. 1A and 1B, and can manage communication and control of the various electronic functions. The main control function can also support electrical and communications interface 908 with a user or an output device such as a printer 920, as well as optional diagnostic and safety functions. In conjunction with main control function 900, multiplexer function 902 can control sensor data 914 and output current 916 to help control heater assembly 977. The display control function 904 can control output to and, if applicable, interpret input from touch screen LCD 846, which can thereby provide a graphical interface to the user in certain embodiments. The detector function 906 can be implemented in control electronics 840 using typical control and processing circuitry to collect, digitize, filter, and/or transmit the data from a detector 999 such as one or more fluorescence detectors. Additional functions, not shown in FIG. 2, include but are not limited to control functions for controlling elements in FIGS. 1A and 1B such as a liquid dispense head, a separator, a cooler, and to accept data from a sample reader.

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An exemplary apparatus, having functions according to FIG. 1A or 1B, is shown in FIGS. 3A and 3B. The exemplary apparatus in FIGS. 3A and 3B has a housing 985, and a cover 987, shown in a closed position in FIG. 3A, and in an open position in FIG. 3B to reveal interior features 995. Cover 987 optionally has a handle 989, shown as oval and raised from the surface of the cover, but which may be other shapes such as square, rectangular, or circular, and which may be recessed in, or flush with, the surface of the cover. Cover 987 is shown as having a hinge, though other configurations such as a sliding cover are possible. Bumper 991 serves to prevent the cover from falling too far backwards and/or provides a point that holds cover 987 steady in an open position. Housing 985 is additionally shown as having one or more communications ports 983, and one or more power ports 993, which may be positioned elsewhere, such as on the rear of the instrument.

The apparatus of FIGS. 1A and 1B may optionally comprise one or more stabilizing feet that cause the body of the device to be elevated above a surface on which system 100 is disposed, thereby permitting ventilation underneath system 100, and also providing a user with an improved ability to lift system 100. There may be 2, 3, 4, 5, or 6, or more feet, depending upon the size of system 100. Such feet are preferably made of rubber, or plastic, or metal, and in some embodiments may elevate the body of system 10 by from about 2 to about 10 mm above a surface on which it is situated.

FIG. 4 shows an exemplary configuration of a portion of an interior of an exemplary apparatus, such as that shown in FIGS. 3A and 3B. In FIG. 4 are shown a rack 970, containing a number of reagent holders 972 and patient samples 996, as well as, in close proximity thereto, a receiving bay 992 having a cartridge 994, for performing PCR on polynucleotides extracted from the samples.

Rack

The apparatus further comprises one or more racks configured to be insertable into, and removable from, the apparatus, each of the racks being further configured to receive a plurality of reagent holders, and to receive a plurality of sample tubes, wherein the reagent holders are in one-to-one correspondence with the sample tubes, and wherein the reagent holders each contain sufficient reagents to extract polynucleotides from a sample and place the polynucleotides into a PCR-ready form. Exemplary reagent holders are further described elsewhere herein.

An apparatus may comprise 1, 2, 3, 4, or 6 racks, and each rack may accept 2, 4, 6, 8, 10, 12, 16, or 20 samples such as in sample tubes 802, and a corresponding number of holders 804, each at least having one or more pipette tips, and one or more containers for reagents.

A rack is typically configured to accept a number of reagent holders 804, such as those further described herein, the rack being configured to hold one or more such holders, either permitting access on a laboratory benchtop to reagents stored in the holders, or situated in a dedicated region of the apparatus permitting the holders to be accessed by one or more other functions of the apparatus, such as automated pipetting, heating of the process tubes, and magnetic separating of affinity beads.

Two perspective views of an exemplary rack 800, configured to accept 12 sample tubes and 12 corresponding reagent holders, in 12 lanes, are shown in FIG. 5. A lane, as used herein in the context of a rack, is a dedicated region of the rack designed to receive a sample tube and correspond-

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ing reagent holder. Two perspective views of the same exemplary rack, in conjunction with a heater unit, are shown in FIG. 6.

Various views of a second exemplary rack 800, also configured to accept 12 sample tubes and 12 reagent holders, are shown in FIG. 7, and FIGS. 8A-8K. Thus, the following views are shown: side plan (FIG. 8A); front plan, showing sample tubes (FIG. 8B); rear plan, showing reagent holders (FIG. 8C); rear elevation, showing reagent holders (FIG. 8D); front elevation, showing sample tubes (FIG. 8E); top, showing insertion of a reagent holder (FIGS. 8F and 8G); top showing slot for inserting a reagent holder (FIG. 8H); top view showing registration of reagent holder (FIG. 8I); close up of rack in state of partial insertion/removal from apparatus (FIG. 8J); and rack held by handle, removed from apparatus (FIG. 8K). A recessed area in a diagnostic or preparatory apparatus, as further described herein, for accepting the exemplary removable rack of FIG. 7 is shown in FIG. 9. Other suitably configured recessed areas for receiving other racks differing in shape, appearance, and form, rather than function, are consistent with the description herein.

The two exemplary racks shown in the figures being non-limiting, general features of racks contemplated herein are now described using the two exemplary racks as illustrative thereof. For example, the embodiments shown here, at least the first lane and the second lane are parallel to one another, a configuration that increases pipetting efficiency. Typically, when parallel to one another, pairs of adjacent sample lanes are separated by 24 mm at their respective midpoints. (Other distances are possible, such as 18 mm apart, or 27 mm apart. The distance between the midpoints in dependent on the pitch of the nozzles in the liquid dispensing head, as further described herein. Keeping the spacing in multiples of 9 mm enables easy loading from the rack into a 96 well plate (where typically wells are spaced apart by 9 mm). Typically, also, the rack is such that plurality of reagent holders in the plurality of lanes are maintained at the same height relative to one another.

The rack is configured to accept a reagent holder in such a way that the reagent holder snaps or locks reversibly into place, and remains steady while reagents are accessed in it, and while the rack is being carried from one place to another or is being inserted into, or removed from, the apparatus. In each embodiment, each of the second locations comprises a mechanical key configured to accept the reagent holder in a single orientation. In FIG. 5, it is shown that the reagent holder(s) slide horizontally into vertically oriented slots, one per holder, located in the rack. In such an embodiment, the edge of a connecting member on the holder engages with a complementary groove in the upper portion of a slot. In FIGS. 8F, 8G, and 8I, it is shown that the reagent holder(s) can engage with the rack via a mechanical key that keeps the holders steady and in place. For example, the mechanical key can comprise a raised or recessed portion that, when engaging with a complementary portion of the reagent holder, permits the reagent holder to snap into the second location. It can also be seen in the embodiments shown that the reagent holder has a first end and a second end, and the mechanical key comprises a first feature configured to engage with the first end, and a second feature configured to engage with the second end in such a way that a reagent holder cannot be inserted the wrong way around.

In certain embodiments the reagent holders each lock into place in the rack, such as with a cam locking mechanism that is recognized as locked audibly and/or physically, or such as with a mechanical key. The rack can be configured so that

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the holders, when positioned in it, are aligned for proper pipette tip pick-up using a liquid dispenser as further described herein. Furthermore, the second location of each lane can be deep enough to accommodate one or more pipette tips, such as contained in a pipette tip sheath.

In certain embodiments, the rack is configured to accept the samples in individual sample tubes **802**, each mounted adjacent to a corresponding holder **804**, for example on one side of rack **800**. The sample tubes can be accessible to a sample identification verifier such as a bar code reader, as further described herein. In FIG. **5**, a sample tube is held at its bottom by a cylindrical receiving member. In FIG. **7**, it is shown that a sample tube can be held at both its top and bottom, such as by a recessed portion **803** configured to receive a bottom of a sample tube, and an aperture **805** configured to hold an upper portion of the sample tube. The aperture can be a ring or an open loop, or a hole in a metal sheet. The recessed portion can be as in FIG. **7**, wherein it is an angled sheet of metal housing having a hole large enough to accommodate a sample tube.

The rack can be designed so that it can be easily removed from the apparatus and carried to and from the laboratory environment external to the apparatus, such as a bench, and the apparatus, for example, to permit easy loading of the sample tube(s) and the reagent holder(s) into the rack. In certain embodiments, the rack is designed to be stable on a horizontal surface, and not easily toppled over during carriage, and, to this end, the rack has one or more (such as 2, 3, 4, 6, 8) feet **809**. In certain embodiments, the rack has a handle **806** to ease lifting and moving, and as shown in FIG. **5**, the handle can be locked into a vertical position, during carriage, also to reduce risk of the rack being toppled over. The handle can optionally have a soft grip **808** in its middle. In the embodiment of FIG. **7**, the carrying handle is positioned about an axis displaced from an axis passing through the center of gravity of the rack when loaded, and is free to fall to a position flush with an upper surface of the rack, under its own weight.

The embodiment of FIG. **5** has a metallic base member **810** having 4 feet **811** that also serve as position locators when inserting the rack into the dedicated portion of the apparatus. The handle is attached to the base member. The portion of the rack **812** that accepts the samples and holders can be made of plastic, and comprises 12 slots, and may be disposable.

In the embodiment of FIG. **7**, the rack comprises a housing, a plurality of lanes in the housing, and wherein each lane of the plurality of lanes comprises: a first location configured to accept a sample tube; and a second location, configured to accept a reagent holder; and a registration member complementary to a receiving bay of a diagnostic apparatus. Typically, the housing is made of a metal, such as aluminum, that is both light but also can be machined to high tolerance and is sturdy enough to ensure that the rack remains stable when located in the diagnostic apparatus. The registration member in FIG. **7** comprises four (4) tight tolerance pegs **815**, located one per corner of the rack. Such pegs are such that they fit snugly and tightly into complementary holes in the receiving bay of the apparatus and thereby stabilize the rack. Other embodiments having, for example, 2, or 3, or greater than 4 such pegs are consistent with the embodiments herein.

In particular, the housing in the embodiment of FIG. **7** comprises a horizontal member **821**, and two or more vertical members **822** connected to the horizontal member, and is such that the second location of each respective lane is a recessed portion within the horizontal member. The two

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or more vertical members **809** in the embodiment of FIG. **7** are configured to permit the rack to free stand thereon. The housing may further comprise two or more feet or runners, attached symmetrically to the first and second vertical members and giving the rack additional stability when positioned on a laboratory bench top.

Furthermore, in the embodiment of FIG. **7**, the housing further comprises a plurality of spacer members **825**, each of which is disposed between a pair of adjacent lanes. Optionally, such spacer members may be disposed vertically between the lanes.

Although not shown in the FIGs., a rack can further comprise a lane identifier associated with each lane. A lane identifier may be a permanent or temporary marking such as a unique number or letter, or can be an RFID, or bar-code, or may be a colored tag unique to a particular lane.

A rack is configured so that it can be easily placed at the appropriate location in the instrument and gives the user positive feedback, such as audibly or physically, that it is placed correctly. In certain embodiments, the rack can be locked into position. It is desirable that the rack be positioned correctly, and not permitted to move thereafter, so that movement of the liquid dispenser will not be compromised during liquid handling operations. The rack therefore has a registration member to ensure proper positioning. In the embodiment of FIG. **7**, the registration member comprises two or more positioning pins configured to ensure that the rack can only be placed in the diagnostic apparatus in a single orientation; and provide stability for the rack when placed in the diagnostic apparatus. The embodiment of FIG. **7** has, optionally, a sensor actuator **817** configured to indicate proper placement of the rack in the diagnostic apparatus. Such a sensor may communicate with a processor **980** to provide the user with a warning, such as an audible warning, or a visual warning communicated via an interface, if the rack is not seated correctly. It may also be configured to prevent a sample preparation process from initiating or continuing if a seating error is detected.

In certain embodiments, the interior of the rack around the location of process tubes in the various holders is configured to have clearance for a heater assembly and/or a magnetic separator as further described herein. For example, the rack is configured so that process chambers on the individual holders are accepted by heater units in a heater assembly as further described herein.

Having a removable rack enables a user to keep a next rack loaded with samples and in line while a previous rack of samples is being prepared by the apparatus, so that the apparatus usage time is maximized.

The rack can also be conveniently cleaned outside of the instrument in case of any sample spills over it or just as a routine maintenance of laboratory wares.

In certain embodiments the racks have one or more disposable parts.

Holder

FIGS. **10A** and **10B** show views of an exemplary holder **501** as further described herein. FIG. **11** shows a plan view of another exemplary holder **502**, as further described herein. FIG. **12A** shows an exemplary holder **503** in perspective view, and FIG. **12B** shows the same holder in cross-sectional view. FIG. **12C** shows an exploded view of the same holder as in FIGS. **12A** and **12B**. All of these exemplary holders, as well as others consistent with the written description herein though not shown as specific embodiments, are now described.

The exemplary holders shown in FIGS. **10A**, **10B**, **11**, **12A**, **12B**, and **12C** can each be referred to as a “unitized

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disposable strip”, or a “unitized strip”, because they are intended to be used as a single unit that is configured to hold all of the reagents and receptacles necessary to perform a sample preparation, and because they are laid out in a strip format. It is consistent with the description herein, though, that other geometric arrangements of the various receptacles are contemplated, so that the description is not limited to a linear, or strip, arrangement, but can include a circular or grid arrangement.

Some of the reagents contained in the holder are provided as liquids, and others may be provided as solids. In some embodiments, a different type of container or tube is used to store liquids from those that store the solids.

The holder can be disposable, such as intended for a single use, following which it is discarded.

The holder is typically made of a plastic such as polypropylene. The plastic is such that it has some flexibility to facilitate placement into a rack, as further described herein. The plastic is typically rigid, however, so that the holder will not significantly sag or flex under its own weight and will not easily deform during routine handling and transport, and thus will not permit reagents to leak out from it.

The holder comprises a connecting member **510** having one or more characteristics as follows. Connecting member **510** serves to connect various components of the holder together. Connecting member **510** has an upper side **512** and, opposed to the upper side, an underside **514**. In FIG. **10B**, a view of underside **514** is shown, having various struts **597** connecting a rim of the connecting member with variously the sockets, process tube, and reagent tubes. Struts **597** are optional, and may be omitted all or in part, or may be substituted by, in all or in part, other pieces that keep the holder together.

The holder is configured to comprise: a process tube **520** affixed to the connecting member and having an aperture **522** located in the connecting member; at least one socket **530**, located in the connecting member, the socket configured to accept a disposable pipette tip **580**; two or more reagent tubes **540** disposed on the underside of the connecting member, each of the reagent tubes having an inlet aperture **542** located in the connecting member; and one or more receptacles **550**, located in the connecting member, wherein the one or more receptacles are each configured to receive a complementary container such as a reagent tube (not shown) inserted from the upper side **512** of the connecting member.

The holder is typically such that the connecting member, process tube, and the two or more reagent tubes are made from a single piece, such as a piece of polypropylene.

The holder is also typically such that at least the process tube, and the two or more reagent tubes are translucent.

The one or more receptacles **550** are configured to accept reagent tubes that contain, respectively, sufficient quantities of one or more reagents typically in solid form, such as in lyophilized form, for carrying out extraction of nucleic acid from a sample that is associated with the holder. The receptacles can be all of the same size and shape, or may be of different sizes and shapes from one another. Receptacles **550** are shown as having open bottoms, but are not limited to such topologies, and may be closed other than the inlet **552** in the upper side of connecting member **510**. Preferably the receptacles **550** are configured to accept commonly used containers in the field of laboratory analysis, or containers suitably configured for use with the holder herein. The containers are typically stored separately from the holders to facilitate sample handling, since solid reagents normally

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require different storage conditions from liquid reagents. In particular many solid reagents may be extremely moisture sensitive.

The snapped-in reagent tubes containing different reagents may be of different colors, or color-coded for easy identification by the user. For example they may be made of different color material, such as tinted plastic, or may have some kind of identifying tag on them, such as a color stripe or dot. They may also have a label printed on the side, and/or may have an identifier such as a barcode on the sealing layer on the top.

The containers **554** received by the receptacles **550** may alternatively be an integrated part of the holder and may be the same type of container as the waste chamber and/or the reagent tube(s), or may be different therefrom.

In one embodiment, the containers **554** containing lyophilized reagents, disposed in the receptacles **550** (shown, e.g., in FIGS. **12A** and **12C**), are 0.3 ml tubes that have been further configured to have a star pattern (see FIGS. **13A** and **13B**) on their respective bottom interior surfaces. This is so that when a fluid has been added to the lyophilized reagents (which are dry in the initial package), a pipette tip can be bottomed out in the tube and still be able to withdraw almost the entire fluid from the tube, as shown in FIG. **14**, during the process of nucleic acid extraction. The design of the star-pattern is further described elsewhere herein.

The reagent tubes, such as containing the lyophilized reagents, can be sealed across their tops by a metal foil, such as an aluminum foil, with no plastic lining layer, as further described herein.

The embodiments **501**, **502**, and **503** are shown configured with a waste chamber **560**, having an inlet aperture **562** in the upper side of the connecting member. Waste chamber **560** is optional and, in embodiments where it is present, is configured to receive spent liquid reagents. In other embodiments, where it is not present, spent liquid reagents can be transferred to and disposed of at a location outside of the holder, such as, for example, a sample tube that contained the original sample whose contents are being analyzed. Waste chamber **560** is shown as part of an assembly comprising additionally two or more reagent tubes **540**. It would be understood that such an arrangement is done for convenience, e.g., of manufacture; other locations of the waste chamber are possible, as are embodiments in which the waste chamber is adjacent a reagent tube, but not connected to it other than via the connecting member.

The holder is typically such that the connecting member, process tube, the two or more reagent tubes, and the waste chamber (if present) are made from a single piece, made from a material such as polypropylene.

The embodiments **501** and **503** are shown having a pipette sheath **570**. This is an optional component of the holders described herein. It may be permanently or removably affixed to connecting member **510**, or may be formed, e.g., moulded, as a part of a single piece assembly for the holder. For example, exploded view of holder **503** in FIG. **12C** shows lug-like attachments **574** on the upper surface of a removable pipette sheath **570** that engage with complementary recessed portions or holes in the underside **514** of connecting member **510**. Other configurations of attachment are possible. Pipette sheath **570** is typically configured to surround the at least one socket and a tip and lower portion of a pipette tip when the pipette tip is stationed in the at least one socket. In some embodiments, the at least one socket comprises four sockets. In some embodiments the at least one socket comprises two, three, five, or six sockets.

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Pipette sheath **570** typically is configured to have a bottom **576** and a walled portion **578** disposed between the bottom and the connecting member. Pipette sheath **570** may additionally and optionally have one or more cut-out portions **572** in the wall **578**, or in the bottom **576**. Such cutouts provide ventilation for the pipette lips and also reduce the total amount of material used in manufacture of the holder. Embodiment **503** has a pipette sheath with no such cutouts. In embodiment **501**, such a cutout is shown as an isosceles triangle in the upper portion of the sheath; a similar shaped cutout may be found at a corresponding position in the opposite side of the sheath, obscured from view in FIG. **10A**. Other cutouts could have other triangular forms, circular, oval, square, rectangular, or other polygonal or irregular shapes, and be several, such as many, in number. The wall **578** of pipette sheath **570** may also have a mesh or frame like structure having fenestrations or interstices. In embodiments having a pipette sheath, a purpose of the sheath is to catch drips from used pipette tips, and thereby to prevent cross-sample contamination, from use of one holder to another in a similar location, and/or to any supporting rack in which the holder is situated. Typically, then, the bottom **576** is solid and bowl-shaped (concave) so that drips are retained within it. An embodiment such as **502**, having no pipette sheath, could utilize, e.g., a drip tray or a drainage outlet, suitably placed beneath pipette tips located in the one or more sockets, for the same purpose. In addition to catching drips, the pipette tip sheath prevents or inhibits the tips of other reagent holders—such as those that are situated adjacent to the one in question in a rack as further described herein—from touching each other when the tips are picked up and/or dropped off before or after some liquid processing step. Contact between tips in adjacent holders is generally not intended by, for example, an automated dispensing head that controls sample processing on holders in parallel, but the pipette tips being long can easily touch a tip in a nearby strip if the angle when dropping off of the tip deviates slightly from vertical.

The holders of embodiments **501**, **502**, and **503**, all have a connecting member that is configured so that the at least one socket, the one or more receptacles, and the respective apertures of the process tube, and the two or more reagent tubes, are all arranged linearly with respect to one another (i.e., their midpoints lie on the same axis). However, the holders herein are not limited to particular configurations of receptacles, waste chamber, process tube, sockets, and reagent tubes. For example, a holder may be made shorter, if some apertures are staggered with respect to one another and occupy 'off-axis' positions. The various receptacles, etc., also do not need to occupy the same positions with respect to one another as is shown in FIGS. **12A** and **12B**, wherein the process tube is disposed approximately near the middle of the holder, liquid reagents are stored in receptacles mounted on one side of the process tube, and receptacles holding solid reagents are mounted on the other side of the process tube. Thus, in FIGS. **10A**, **10B**, and **11**, the process tube is on one end of the connecting member, and the pipette sheath is at the other end, adjacent to, in an interior position, a waste chamber and two or more reagent tubes. Still other dispositions are possible, such as mounting the process tube on one end of the holder, mounting the process tube adjacent the pipette tips and pipette tip sheath (as further described herein), and mounting the waste tube adjacent the process tube. It would be understood that alternative configurations of the various parts of the holder give rise only to variations of form and can be accommodated within other variations of the apparatus as described, including but not limited to

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alternative instruction sets for a liquid dispensing pipette head, heater assembly, and magnetic separator, as further described herein.

Process tube **520** can also be a snap-in tube, rather than being part of an integrated piece. Process tube **520** is typically used for various mixing and reacting processes that occur during sample preparation. For example, cell lysis can occur in process tube **520**, as can extraction of nucleic acids. Process tube **520** is then advantageously positioned in a location that minimizes, overall, pipette head moving operations involved with transferring liquids to process tube **520**.

Reagent tubes **540** are typically configured to hold liquid reagents, one per tube. For example, in embodiments **501**, **502**, and **503**, three reagent tubes are shown, containing respectively wash buffer, release buffer, and neutralization buffer, each of which is used in a sample preparation protocol.

Reagent tubes **540** that hold liquids or liquid reagents can be sealed with a laminate structure **598**. The laminate structure typically has a heat seal layer, a plastic layer such as a layer of polypropylene, and a layer of metal such as aluminum foil, wherein the heat seal layer is adjacent the one or more reagent tubes. The additional plastic film that is used in a laminate for receptacles that contain liquid reagents is typically to prevent liquid from contacting the aluminum.

Two embodiments of a laminate structure, differing in their layer structures, are shown in FIG. **15**. In both embodiments, the heat seal layer **602**, for example made of a laquer or other such polymer with a low melting point, is at the bottom, adjacent to the top of the holder, when so applied. The plastic layer **604** is typically on top of the heat seal layer, and is typically made of polypropylene, having a thickness in the range 10-50 microns. The metal layer **608** is typically on top of the plastic layer and may be a layer of Al foil bonded to the plastic layer with a layer of adhesive **606**, as in the first embodiment in FIG. **15**, or may be a layer of metal that is evaporated or sputtered into place directly on to the plastic layer. Exemplary thicknesses for the respective layers are shown in FIG. **15**, where it is to be understood that variations of up to a factor of 2 in thickness are consistent with the technology herein. In particular, the aluminum foil is 0.1-15 microns thick, and the polymer layer is 15-25 microns thick in one embodiment. In another embodiment, the aluminum is 0.1-1 microns thick, and the polymer layer is 25-30 microns thick.

The laminates deployed herein make longer term storage easier because the holder includes the presence of sealed lyophilized reagents as well as liquids sealed in close proximity, which is normally hard to achieve.

In one embodiment, the tops of the reagent tubes have beveled edges so that when an aluminum foil is heat bonded to the top, the plastic melt does not extend beyond the rim of the tube. This is advantageous because, if the plastic melt reduces the inner diameter of the tube, it will cause interference with the pipette tip during operation. In other embodiments, a raised flat portion **599** facilitates application and removal of laminate **598**. Raised surface **599**, on the upper side of the connecting member, and surrounding the inlet apertures to the reagent tubes and, optionally, the waste chamber, is an optional feature of the holder.

The manner in which liquid is pipetted out is such that a pipette tip piercing through the foil rips through without creating a seal around the pipette tip, as in FIG. **16**. Such a seal around the tip during pipetting would be disadvantageous because a certain amount of air flow is desirable for the pipetting operation. In this instance, a seal is not created

because the laminate structure causes the pierced foil to stay in the position initially adopted when it is pierced. The upper five panels in FIG. 16 illustrate the pipetting of a reagent out from a reagent tube sealed with a laminate as further described herein. At A, the pipette tip is positioned approximately centrally above the reagent tube that contains reagent 707. At B, the pipette tip is lowered, usually controllably lowered, into the reagent tube, and in so doing pierces the foil 598. The exploded view of this area shows the edge of the pierced laminate to be in contact with the pipette tip at the widest portion at which it penetrates the reagent tube. At C, the pipette tip is withdrawn slightly, maintaining the tip within the bulk of the reagent 707. The exploded view shows that the pierced foil has retained the configuration that it adopted when it was pierced and the pipette tip descended to its deepest position within the reagent tube. At D, the pipette tip sucks up reagent 707, possibly altering its height as more and more older people undergo such tests. At E, the pipette tip is removed entirely from the reagent tube.

The materials of the various tubes and chambers may be configured to have at least an interior surface smoothness and surface coating to reduce binding of DNA and other macromolecules thereto. Binding of DNA is unwanted because of the reduced sensitivity that is likely to result in subsequent detection and analysis of the DNA that is not trapped on the surface of the holder.

The process tube also may have a low binding surface, and allows magnetic beads to slide up and down the inside wall easily without sticking to it. Moreover, it has a hydrophobic surface coating enabling low stiction of fluid and hence low binding of nucleic acids and other molecules.

In some embodiments, the holder comprises a registration member such as a mechanical key. Typically such a key is part of the connecting member 510. A mechanical key ensures that the holder is accepted by a complementary member in, for example, a supporting rack or a receiving bay of an apparatus that controls pipetting operations on reagents in the holder. A mechanical key is normally a particular-shaped cut-out that matches a corresponding cutout or protrusion in a receiving apparatus. Thus, embodiment 501 has a mechanical key 592 that comprises a pair of rectangular-shaped cut-outs on one end of the connecting member. This feature as shown additionally provides for a tab by which a user may gain a suitable purchase when inserting and removing the holder into a rack or another apparatus. Embodiments 501 and 502 also have a mechanical key 590 at the other end of connecting member 510. Key 590 is an angled cutout that eases insertion of the holder into a rack, as well as ensures a good registration therein when abutting a complementary angled cut out in a recessed area configured to receive the holder. Other variations of a mechanical key are, of course, consistent with the description herein: for example, curved cutouts, or various combinations of notches or protrusions all would facilitate secure registration of the holder.

In some embodiments, not shown in FIG. 10A, 10B, 11, or 12A-C, the holder further comprises an identifier affixed to the connecting member. The identifier may be a label, such as a writable label, a bar-code, a 2-dimensional bar-code, or an RFID tag. The identifier can be, e.g., for the purpose of revealing quickly what combination of reagents is present in the holder and, thus, for what type of sample preparation protocol it is intended. The identifier may also indicate the batch from which the holder was made, for quality control or record-keeping purposes. The identifier may also permit a user to match a particular holder with a particular sample.

It should also be considered consistent with the description herein that a holder additionally can be configured to accept a sample, such as in a sample tube. Thus, in embodiments described elsewhere herein, a rack accepts a number of sample tubes and a number of corresponding holders in such a manner that the sample tubes and holders can be separately and independently loaded from one another. Nevertheless, in other embodiments, a holder can be configured to also accept a sample, for example in a sample tube. And thus, a complementary rack is configured to accept a number of holders, wherein each holder has a sample as well as reagents and other items. In such an embodiment, the holder is configured so that the sample is accessible to a sample identification verifier.

Kits

The holder described herein may be provided in a sealed pouch, to reduce the chance of air and moisture coming into contact with the reagents in the holder. Such a sealed pouch may contain one or more of the holders described herein, such as 2, 4, 6, 8, 10, 12, 16, 20, or 24 holders.

The holder may also be provided as part of a kit for carrying out sample preparation, wherein the kit comprises a first pouch containing one or more of the holders described herein, each of the holders configured with liquid reagents for, e.g., lysis, wash, and release, and a second pouch, having an inert atmosphere inside, and one or more reagent tubes containing lyophilized PCR reagents, as shown in FIG. 17. Such a kit may also be configured to provide for analysis of multiple samples, and contain sufficient PCR reagents (or other amplification reagents, such as for RT-PCR, transcription mediated amplification, strand displacement amplification, NASBA, helicase dependent amplification, and other familiar to one of ordinary skill in the art, and others described herein) to process such samples, and a number of individual holders such as 2, 4, 6, 8, 10, 12, 16, 20, or 24 holders.

Reagent Tubes

As referenced elsewhere herein, the containers 554 that contain lyophilized reagents are 0.3 ml tubes that have been further configured to have a star-shaped—or stellated—pattern (see FIGS. 13A and 13B) on their respective bottom interior surfaces. Still other tubes for use herein, as well as for other uses not herein described, can be similarly configured. Thus, for example, the benefits afforded by the star-shaped pattern also accrue to reagent tubes that contain liquid samples that are directly pipetted out of the tubes (as well as to those tubes that initially hold solids that are constituted into liquid form prior to pipetting). Other size tubes that would benefit from such a star-shaped pattern have sizes in the range 0.1 ml to 0.65 ml. for example.

The star-shaped pattern ensures that when a fluid is withdrawn from the tube, a pipette tip can be bottomed out in the tube and still be able to withdraw the entire, or almost the entire fluid from the tube, as shown in FIG. 14. This is important because, when working with such small volumes, and when target DNA can be present in very few copies, sample loss due to imperfections of pipetting is to be minimized to every extent possible.

The design of the star shaped pattern is important, especially when using for recovery of DNA/RNA present in very small numbers in the clinical sample. The stellated pattern should enable pipetting of most of the liquid (residual volume < 1 microliter) when used with a pipette bottomed out with the bottom of the tube. Additionally, the stellated pattern should be designed to minimize surface area as well as dead-end grooves that tend to have two undesirable

effects—to trap liquid as well as to increase undesirable retention of polynucleotides by adsorption.

FIG. 14 is now described, as follows. FIG. 14 has a number of panels, A-G, each representing, in sequence, a stage in a pipetting operation. At A, a pipette tip 2210, containing a liquid 2211 (such as a buffer solution), is positioned directly or approximately above the center of reagent tube 2200. The tube contains a number of lyophilized pellets 2212, and is sealed by a layer 2214, such as of foil. The foil may be heat-sealed on to the top of the tube. Although a laminate layer, as further described herein, can be placed on the reagent tube, typically a layer of aluminum foil is adequate, where the tube contents are solid, e.g., lyophilized, reagents. In some embodiments, the top of the reagent tube has chamfer edges to reduce expansion of the top rim of the tube during heat sealing of a foil on the top of the tube. The tube may further comprise an identifiable code, such as a 1-D or a 2-D bar-code on the top. Such a code is useful for identifying the composition of the reagents stored within, and/or a batch number for the preparation thereof, and/or an expiry date. The code may be printed on with, for example, an inkjet or transfer printer.

Stellated pattern 2203 on the bottom interior surface of the tube 2200 is shown. At B, the pipette tip is lowered, piercing seal 2214, and brought into a position above the particles 2212. At C the liquid 2211 is discharged from the pipette tip on to the particles, dissolving the same, as shown at D. After the particles are fully dissolved, forming a solution 2218, the pipette tip is lowered to a position where it is in contact with the stellated pattern 2203. At E, the pipette tip is caused to suck up the solution 2218, and at F, the tip may optionally discharge the solution back into the tube. Steps E and F may be repeated, as desired, to facilitate dissolution and mixing of the lyophilized components into solution. At step G, after sucking up as much of the solution 2218 as is practicable into the pipette tip, the pipette tip is withdrawn from the tube. Ideally, 100% by volume of the solution 2218 is drawn up into the pipette tip at G. In other embodiments, and depending upon the nature of solution 2218, at least 99% by volume of the solution is drawn up. In still other embodiments, at least 98%, at least 97%, at least 96%, at least 95%, and at least 90% by volume of the solution is drawn up.

The design of the stellated or star-shaped pattern can be optimized to maximize the flow rate of liquid through the gaps in-between a bottomed out pipette, such as a p1000 pipette, and the star pattern, and is further described in U.S. provisional patent application Ser. No. 60/959,437, filed Jul. 13, 2007, incorporated herein by reference. It would be understood that, although the description herein pertains to pipettes and pipette tips typically used in sample preparation of biological samples, the principles and detailed aspects of the design are as applicable to other types of pipette and pipette tip, and may be so-adapted.

FIG. 13A shows a cross sectional perspective view of a reagent tube 2200 having side wall 2201 and bottom 2202. Interior surface 2204 of the bottom is visible. A star-shaped cutout 2203 is shown in part, as three apical grooves.

Typically the star-shaped pattern is present as a raised portion on the lower interior surface of the tube. Thus, during manufacture of a reagent tube, such as by injection moulding, an outer portion of the mould is a cavity defining the exterior shape of the tube. An interior shape of the tube is formed by a mould positioned concentrically with the outer portion mould, and having a star-shaped structure milled out of its tip. Thus, when liquid plastic is injected into

the space between the two portions of the mould, the star-shape is formed as a raised portion on the bottom interior surface of the tube.

The exemplary star pattern 2203 shown in FIG. 13B in plan view resembles a “ship’s wheel” and comprises a center 2209, a circular ring 2207 centered on center 2209, and 8 radial segments configured as radial grooves 2205. Each groove meets the other grooves at center 2209, and has a radial end, also referred to as an apex or vertex. Star pattern 2203 has 8 grooves, but it would be understood that a star pattern having fewer or a greater number of grooves, such as 3, 4, 6, 10, or 12, would be consistent with the design herein. The number of grooves of the star should be minimum consistent with effective liquid pipetting and also spaced apart enough not to trap the tip of any of the pipette tips to be used in the liquid handling applications.

Center 2209 is typically positioned coincidentally with the geometric center of the bottom of reagent tube 2200. The tube is typically circular in cross-section, so identifying its center (e.g., at a crossing point of two diameters) is normally straightforward. Center 2209 may be larger than shown in FIG. 13B, such as may be a circular cutout or raised portion that exceeds in diameter of the region formed by the meeting point of grooves 2205.

Ring 2207 is an optional feature of star-shaped pattern 2203. Typically ring 2207 is centered about center 2209, and typically it also has a dimension that corresponds to the lower surface of a pipette tip. Thus, when a pipette tip ‘bottoms out’ in the bottom of reagent tube 2200, the bottom of the pipette tip rests in contact with ring 2207. Ring 2207 is thus preferably a cut-out or recessed feature that can accommodate the pipette tip and assist in guiding its positioning centrally at the bottom of the tube. In other embodiments more than one, such as 2, 3, or 4 concentric rings 2207 are present.

The star pattern is configured to have dimensions that give an optimal flow-rate of liquid out of the reagent tube into a suitably positioned pipette tip. The star pattern is shown in FIG. 13B as being significantly smaller in diameter than the diameter of the tube at its widest point. The star pattern may have, in various embodiments, a diameter (measured from center 2209 to apex of a groove 2205) from 5-20% of the diameter of the reagent tube, or from 10-25% of the diameter of the reagent tube, or from 15-30% of the diameter of the reagent tube, or from 20-40% of the diameter of the reagent tube, or from 25-50% of the diameter of the reagent tube, or from 30-50% the diameter of the reagent tube, or from 40-60% the diameter of the reagent tube, or from 50-75% the diameter of the reagent tube, or from 65-90% the diameter of the reagent tube.

The grooves 2205 are thus separated by ridges (occupying the space in between adjacent grooves). In the embodiment shown, the grooves are narrower (occupy a smaller radial angle) than the gaps between them. In other embodiments, the grooves may be proportionately wider than the gaps between them. In such embodiments, it may be more appropriate to describe them as having ridges instead of grooves. In other embodiments, the grooves and ridges that separate them are of equal widths at each radial distance from the center.

The grooves that form the apices of the star may be rounded in their lower surfaces, such as semi-circular in cross section, but are typically V-shaped. They may also be trapezoid in cross-section, such as having a wider upper portion than the bottom, which is flat, the upper portion and the bottom being connected by sloping walls.

In some embodiments, for ease of manufacture, the grooves end on the same level in the bottom of the tube. Thus the radial ends are all disposed on the circumference of a circle. In other embodiments, the grooves do not all end on the same level. For example, grooves may alternately end on different levels, and thus the ends are alternately disposed on the respective circumferences of two circles that occupy different planes in space from one another.

Grooves **2205** are shown in FIG. **13B** as having equal lengths (as measured from center **2209** to apex). This need not be so. In alternative embodiments, grooves may have different lengths from one another, for example, as alternating lengths on alternating grooves, where there are an even number of grooves. Furthermore, apices may be rounded, rather than pointed.

Typically the grooves taper uniformly in width and depth from center **2209** to each respective apex. Still other configurations are possible, such as a groove that follows a constant width, or depth, out to a particular radial extent, such as 30-60% of its length, and then narrows or becomes shallower towards its apex. Alternatively, a groove may start narrow at center **2209**, widen to a widest region near its midpoint of length, and then narrow towards its apex. Still other possibilities, not described herein, are consistent with the stellated pattern.

In a 0.3 ml tube, the width of each groove **2205** at its widest point is typically around 50 microns, and the width typically tapers uniformly from a widest point, closest to or at center **2209**, to the apex.

In a 0.3 ml tube, the depth of a groove at the deepest point is typically around 25-50 microns and the depth typically tapers uniformly from a deepest point, closest to or at center **2209**, to an apex.

In a 0.3 ml tube, the radius of the star formed from the grooves, measured as the shortest distance from center **2209** to apex, is typically around 0.5 mm, but may be from 0.1-1 mm, or from 0.3-2 mm.

In another embodiment, in a 0.3 ml tube, the grooves should be rounded off and less than 100 microns deep, or less than 50 microns deep, or less than 25 microns deep.

The stellated pattern typically has a rotation axis of symmetry, the axis disposed perpendicular to the bottom of the tube and through center **2209**, so that the grooves are disposed symmetrically about the rotation axis. By this is meant that, for n grooves, a rotation of $2\pi/n$ about the central (rotational) axis can bring each groove into coincidence with the groove adjacent to it.

The stellated shape shown in FIG. **13B** is not limiting in that it comprises a number of radially disposed grooves **2205**, and an optional circular ring **2207**. Other star-shaped geometries may be used, and, depending upon case of manufacture, may be preferred. For example, a star can be created simply by superimposing two or more polygons having a common center, but offset rotationally with respect to one another about the central axis. (See, for example "star polygons" described at the Internet site mathworld.wolfram.com/StarPolygon.html.) Such alternative manners of creating star-shaped patterns are utilizable herein.

Liquid Dispenser

In various embodiments, preparation of a PCR-ready sample for use in subsequent diagnosis using the apparatus as further described herein, can include one or more of the following steps: contacting a neutralized polynucleotide sample with a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides (in some embodiments, the PCR reagent mixture can further include a positive control plasmid and a fluorogenic hybridization

probe selective for at least a portion of the plasmid); in some embodiments, the PCR reagent mixture can be in the form of one or more lyophilized pellets, as stored in a receptacle on a holder, and the method can further include reconstituting the PCR pellet with liquid to create a PCR reagent mixture solution. Various, such as one or more, of the liquid transfer operations associated with the foregoing steps can be accomplished by an automated pipette head.

A suitable liquid dispenser for use with the apparatus herein comprises one or more sensors; a manifold; one or more pumps in fluid communication with the manifold; one or more dispense heads in fluid communication with the manifold; and electrical connections that accept electrical signals from an external controller, wherein the liquid dispenser has no inlet or outlet for fluids, other than through the one or more pumps.

A cross-sectional view of an exemplary liquid dispenser is shown in FIG. **18**. The liquid dispenser is configured to carry out fluid transfer operations on two or more holders simultaneously. As shown in FIG. **18**, liquid dispenser **2105** can be mounted on a gantry having three degrees of translational freedom. Further embodiments can comprise a gantry having fewer than three degrees of translational freedom. The manner of mounting can be by a mechanical fastening such as one or more screws, as shown on the left hand side of FIG. **18**. A suitable gantry comprises three axes of belt-driven slides actuated by encoded stepper motors. The gantry slides can be mounted on a framework of structural angle aluminum or other equivalent material, particularly a metal or metal alloy. Slides aligned in x- and y-directions (directed out of and in the plane of FIG. **18** respectively) facilitate motion of the gantry across an array of holders, and in a direction along a given holder, respectively.

The z-axis of the gantry can be associated with a variable force sensor which can be configured to control the extent of vertical motion of the head during tip pick-up and fluid dispensing operations. Shown in FIG. **18**, for example, a pipette head **1803** can be mounted such that a force acting upwardly against the head can be sensed through a relative motion between the head and a force sensor. For example, when pipette head **1803** forces against a disposable pipette in the rack below it, an upward force is transmitted causing head **1803** to torque around pivot point **2102**, causing set screw **2104** to press against a force sensor. In turn, the force sensor is in communication with a processor or controller that controls at least the vertical motion of the liquid dispenser so that, thereby, the processor or controller can send instructions to arrest the vertical motion of the liquid dispenser upon receiving an appropriate signal from the force sensor. An exemplary force sensor suitable for use herein is available from Honeywell; its specification is shown in an appendix hereto. The force sensor mechanism shown in FIG. **18** is exemplary and one of many possible mechanisms capable of commanding the head during up pick-up and fluid dispensing operations. For example, as an alternative to a force sensor, a stall sensor that senses interruption in vertical motion of the one or more dispense heads upon contact with a sample tube or reagent holder may be used. Accordingly, as would be understood by one of ordinary skill in the art, the liquid dispenser as described herein is not limited to the specific mechanism shown in FIG. **18**.

The liquid dispenser further comprises a number of individually sprung heads **1803**, wherein each head is configured to accept a pipette tip from the one or more pipette tips in a holder. The liquid dispenser can be further configured such that no two heads accept pipette tips from the same

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holder. FIGS. 19A-C, for example, depicts four individually sprung heads **1803**, but it is to be understood that the dispenser is not limited to this number. For example, other numbers include 2, 3, 5, 6, 8, 10, or 12. Furthermore, the individually sprung heads **1803** are shown arranged in parallel to one another, but may be configured in other arrangements.

The liquid dispenser can further comprise computer-controlled pump **2100** connected to distribution manifold **1802** with related computer controlled valving. Distribution manifold **1802** can comprise a number of valves, such as solenoid valves **1801** configured to control the flow of air through the pipette tips: in an exemplary embodiment, there are two valves for each pipette, and one additional valve to vent the pump. Thus, for a liquid dispenser having four pipette heads, there are nine valves. In another embodiment there is only one valve for each pipette, and one additional valve to vent the pump. However, the distribution manifold is not limited to comprising exactly nine solenoid valves.

The liquid dispenser is further configured to aspirate or dispense fluid in connection with analysis or preparation of solutions of two or more samples. The liquid dispenser is also configured to dispense liquid into a microfluidic cartridge. Additionally, the liquid dispenser is configured to accept or dispense, in a single operation, an amount of 1.0 ml of fluid or less, such as an amount of fluid in the range 10 nl-1 ml.

The liquid dispenser is configured such that pump **2100** pumps air in and out of the distribution manifold. The distribution manifold comprises a microfluidic network that distributes air evenly amongst the one or more valves. Thus, by controlling flow of air through the manifold and various valves, pressure above the pipette heads can be varied so that liquid is drawn up into or expelled from a pipette tip attached to the respective pipette heads. In this way it is not necessary to supply compressed air via an air hose to the liquid dispenser. Neither is it necessary to provide liquid lines to the dispense head. Furthermore, no liquid reagents or liquid samples from the holders enters any part of the liquid dispenser, including the manifold. This aspect reduces complications from introducing air bubbles into samples or liquid reagents. An exemplary configuration of a distribution manifold is shown in FIG. 20.

As shown in the various figures, the entire liquid dispenser that moves up and down the z-axis is a self-contained unit having only electrical connections to a processor or controller, and mechanical connections to the gantry. The translational motions in three dimensions of the liquid dispenser can be controlled by a microprocessor, such as processor **980**. No fluid handling lines are associated with the dispenser. This design enables simplification of assembly of the instrument, minimizes contamination of the instrument and cross-contamination of samples between different instances of operation of the apparatus, increases efficiency of pumping (minimal dead volume) and enables easy maintenance and repair of the device. This arrangement also enables easy upgrading of features in the dispensing device, such as individual and independent pump control for each dispenser, individual pipette attachment or removal, ability to control the pitch of the pipettes, etc.

Another aspect of the apparatus relates to a sample identification verifier configured to check the identity of each of the number of nucleic-acid containing samples. Such sample identification verifiers can be optical character readers, bar code readers, or radio frequency tag readers, or other suitable readers, as available to one of ordinary skill in the art. A sample identification verifier can be mounted on the gantry, or attached to the liquid dispenser so that it moves in

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concert with the liquid dispenser. Alternatively, the sample identification verifier can be separately mounted and can move independently of the liquid dispenser. In FIGS. 21 and 22, for example, sample identification verifier **1701** is a bar-code reader attached to the liquid dispenser. The field of view of barcode scanner **1701** is non-linear, enabling it to detect light reflected by mirror **2300** from the barcoded clinical sample tube **2301** in disposable rack **2302**. The barcode scanner reads the barcode on the clinical sample tube thus identifying the presence and specifics of the sample tube. Because of use of a mirror, the scanner is configured either to read a bar-code printed in mirror image form (that is thus reflected into normal form), or to read a mirror image of a normal bar-code and to convert the mirror image to unreflected form via a computer algorithm.

Sample identification verifier is configured to communicate details of labels that it has detected or read to a processor or controller in the apparatus, thereby permitting sample identifying information to be associated with diagnostic results and other information relating to sample preparation, and extraction and amplification of nucleic acid therein.

In FIG. 23, the sample identification verifier is positioned to read indicia from a microfluidic cartridge.

In certain embodiments, the liquid dispenser can also comprise one or more sensors **2001** (e.g., infra-red sensors) each of which detects the presence of a pipette tip in a rack. In FIG. 24, for example, an infra-red sensor **2001** can have an infra-red emitter placed opposed to it, and the presence of disposable pipette tip **2000** obstructs the line of sight between the emitter and the detector, thus enabling determination of the presence or absence of the pipette tip. The disposal pipettes are configured perpendicular to pipette stripper-alignment plate **2003** as further described herein.

The liquid dispenser can also operate in conjunction with a motorized plate configured to strip the pipettes and align the pipettes during dispensing of fluid into a microfluidic cartridge, as further described herein.

FIGS. 25A and 25B show an exemplary device for stripping pipette tips from a liquid dispenser as further described herein. The pipette tips are aligned, all at the same pitch, above respective sockets (over a pipette tip sheath) in a holder. A metal plate having elongated holes lies over the sockets. The pipette tips are inserted part way down into the sheath through the elongated holes, and the metal plate is moved along in such a manner that the pipette tips are clamped by the elongated portion of the holes. When the liquid dispenser is moved up, the pipette tips become detached from their respective heads. When the metal plate is subsequently moved back to its initial position, the pipette tips remain in place in their respective sockets.

Heater Assembly & Magnetic Separator

A cross-sectional view of a heater unit of an exemplary heater assembly **1401** is shown in FIG. 18 (right hand panel). The heater assembly comprises one or more independently controllable heater units, each of which comprises a heat block. In certain embodiments there are 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 25, 30, 32, 36, 40, 48, or 50 heater units in a heater assembly. Still other numbers of heater units, such as any number between 6 and 100 are consistent with the description herein. The one or more heat blocks may be fashioned from a single piece of metal or other material, or may be made separately from one another and mounted independently of one another or connected to one another in some way. Thus, the term heater assembly connotes a collection of heater units but does not require the heater units or their respective heat blocks to be attached directly or

indirectly to one another. The heater assembly can be configured so that each heater unit independently heats each of the one or more process tubes **1402**, for example by permitting each of the one or more heat blocks to be independently controllable, as further described herein. In the configuration of FIG. **26**, the heater assembly comprises one or more heat blocks **1403** each of which is configured to align with and to deliver heat to a process tube **1402**. Each heat block **1403** can be optionally secured and connected to the rest of the apparatus using a strip **1408** and one or more screws **1407** or other adhesive device. This securing mechanism is not limited to such a configuration.

Although a cross-sectional view of one heat block **1403** is shown in FIG. **26**, it should be understood that this is consistent with having multiple heat blocks aligned in parallel to one another and such that their geometric mid-points all lie on a single linear axis, though it is not so limited in configuration. Thus, the one or more heat blocks may be positioned at different heights from one another, in groups or, alternately, individually, or may be staggered with respect to one another from left to right in FIG. **26** (right hand panel), in groups or alternately, or individually. Additionally, and in other embodiments, the heat blocks are not aligned parallel to one another but are disposed at angles relative to one another, the angles being other than 180° . Furthermore, although the heat block shown in FIG. **26** may be one of several that are identical in size, it is consistent with the technology herein that one or more heat blocks may be configured to accept and to heat process tubes of different sizes.

The exemplary heat block **1403** in FIG. **26** (right hand panel) is configured to have an internal cavity that partially surrounds a lower portion of process tube **1402**. In the heat block of FIG. **26**, the internal cavity surrounds the lower portion of process tube **1402** on two sides but not the front side (facing away from magnet **1404**) and not the rear side (adjacent to magnet **1404**). In other embodiments, heat block **1403** is configured to surround the bottom of process tube **1402** on three sides, including the front side. Still other configurations of heat block **1403** are possible, consistent with the goals of achieving rapid and uniform heating of the contents of process tube **1402**. In certain embodiments, the heat block is shaped to conform closely to the shape of process tube **1402** so as to increase the surface area of the heat block that is in contact with the process tube during heating of the process tube. Thus, although exemplary heat block **1403** is shown having a conical, curve-bottomed cavity in which a complementary process tube is seated, other embodiments of heat block **1403** have, for example, a cylindrical cavity with a flat bottom. Still other embodiments of heat block **1403** may have a rectilinear internal cavity such as would accommodate a cuvette.

Moreover, although heat block **1403** is shown as an L-shape in FIG. **26**, which aids in the transmittal of heat from heating element **1501** and in securing the one or more heat blocks to the rest of the apparatus, it need not be so, as further described herein. For example, in some embodiments heating element **1501** may be positioned directly underneath process tube **1402**.

Each heat block **1403** is configured to have a low thermal mass while still maintaining high structural integrity and allowing a magnet to slide past the heat blocks and the process tubes with ease. A low thermal mass is advantageous because it allows heat to be delivered or dissipated rapidly, thus increasing the heating and cooling efficiency of the apparatus in which the heater assembly is situated. Factors that contribute to a low thermal mass include the material

from which a heat block is made, and the shape that it adopts. The heat blocks **1403** can therefore be made of such materials as aluminum, silver, gold, and copper, and alloys thereof, but are not so limited.

In one embodiment, the heat block **1403** has a mass of ~10 grams and is configured to heat up liquid samples having volumes between 1.2 ml and 10 μ l. Heating from room temperature to 65° C. for a 1 ml biological sample can be achieved in less than 3 minutes, and 10 μ l of an aqueous liquid such as a release buffer up to 85° C. (from 50° C.) in less than 2 minutes. The heat block **1403** can cool down to 50° C. from 85° C. in less than 3 minutes. The heat block **1403** can be configured to have a temperature uniformity of $65 \pm 4^\circ$ C. for heating up 1 ml of sample and $85 \pm 3^\circ$ C. for heating up 10 μ l of release buffer. These ranges are typical, but the heat block can be suitably scaled to heat other volumes of liquid at rates that are slower and faster than those described. This aspect of the technology is one aspect that contributes to achieving rapid nucleic acid extraction of multiple samples by combination of liquid processing steps, rapid heating for lysis, DNA capture and release and magnetic separation, as further described herein.

Not shown in FIG. **26**, the heater assembly **1401** can also optionally be contained in an enclosure that surrounds the heat blocks **1403**. The enclosure can be configured to enable sufficient air flow around the process tubes and so as not to significantly inhibit rate of cooling. The enclosure can have a gap between it and the heat blocks to facilitate cooling. The enclosure can be made of plastic, but is not so limited. The enclosure is typically configured to appear aesthetic to a user.

As shown in FIG. **26**, the heater assembly **1401** can also comprise one or more heating elements (e.g., a power resistor) **1501** each of which is configured to thermally interface to a heat block **1403** and dissipate heat to it. For example, in one embodiment, a power resistor can dissipate up to 25 Watts of power. A power resistor is advantageous because it is typically a low-cost alternative to a heating element. Other off-the-shelf electronic components such as power transistors may also be used to both sense temperature and heat. Although the heating element **1501** is shown placed at the bottom of the heat block **1403**, it would be understood that other configurations are consistent with the assembly described herein: for example, the heating element **1501** might be placed at the top or side of each heat block **1403**, or directly underneath process tube **1402**. In other embodiments, the heating element has other shapes and is not rectangular in cross section but may be curved, such as spherical or ellipsoidal. Additionally, the heating element may be moulded or shaped so that it conforms closely or approximately to the shape of the bottom of the process tube. Not shown in FIG. **26**, the heater assembly can also comprise an interface material (e.g., Berquist q-pad, or thermal grease) between the heating element **1501** and the heat block **1403** to enable good thermal contact between the element and the heat block.

In the embodiment shown in FIG. **26**, the heater assembly further comprises one or more temperature sensors **1502**, such as resistive temperature detectors, to sense the respective temperatures of each heat block **1403**. Although a temperature sensor **1502** is shown placed at the bottom of the heat block **1403**, it would be understood that other configurations are consistent with the assembly described herein: for example, the temperature sensor might be placed at the top or side of each heat block **1403**, or closer to the bottom of process tube **1402** but not so close as to impede uniform heating thereof. As shown in the embodiment of

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FIG. 26, the heater assembly can further comprise an interface material (e.g., Berquist q-pad) **1503** configured to enable good thermal contact between the sensor **1502** and the heat block **1403**, to thereby ensure an accurate reading.

Certain embodiments of the diagnostic or preparatory apparatus herein have more than one heater assembly as further described herein. For example, a single heater assembly may be configured to independently heat 6 or 12 process tubes, and an apparatus may be configured with two or four such heater assemblies.

The disclosure herein further comprises a magnetic separator, configured to separate magnetic particles, the separator comprising: one or more magnets affixed to a supporting member; a motorized mechanism configured to move the supporting member in such a manner that the one or more magnets move backwards and forwards along a fixed axis, and during at least a portion of the motion, the one or more magnets maintain close proximity to one or more receptacles which contain the magnetic particles in solution; and control circuitry to control the motorized mechanism.

The disclosure herein still further includes an integrated magnetic separator and heater, comprising: a heater assembly, wherein the heater assembly comprises a plurality of independently controllable heater units, each of which is configured to accept and to heat one of a plurality of process tubes; one or more magnets affixed to a supporting member; a motorized mechanism configured to move the supporting member in such a manner that the one or more magnets move backwards and forwards along a fixed axis, and during at least a portion of the motion the one or more magnets maintain close proximity to one or more of the process tubes in the heater assembly, wherein the one or more process tubes contain magnetic particles; and control circuitry to control the motorized mechanism and to control heating of the heater units.

Typically, each of the one or more receptacles is a process tube, such as for carrying out biological reactions. In some embodiments, close proximity can be defined as a magnet having a face less than 2 mm away from the exterior surface of a process tube without being in contact with the tube. It can still further be defined to be less than 1 mm away without being in contact with the tube, or between 1 and 2 mm away.

Typically the magnetic particles are microparticles, beads, or microspheres capable of binding one or more biomolecules, such as polynucleotides. Separating the particles, while in solution, typically comprises collecting and concentrating, or gathering, the particles into one location in the inside of the one or more receptacles.

An exemplary magnetic separator **1400** is shown in FIG. 27, configured to operate in conjunction with heater assembly **1401**. The magnetic separator **1400** is configured to move one or more magnets relative to the one or more process tubes **1402**. While the magnet **1404** shown in FIG. 27 is shown as a rectangular block, it is not so limited in shape. Moreover, the configuration of FIG. 27 is consistent with either having a single magnet that extends across all heat blocks **1403** or having multiple magnets operating in concert and aligned to span a subset of the heat blocks, for example, aligned collinearly on the supporting member. The magnet **1404** can be made of neodymium (e.g., from K & J Magnetics, Inc.) and can have a magnetic strength of 5,000-15,000 Gauss (Brmax). The poles of the magnets **1404** can be arranged such that one pole faces the heat blocks **1403** and the other faces away from the heat blocks.

Further, in the embodiment shown in FIG. 27, the magnet **1404** is mounted on a supporting member **1505** that can be

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raised up and down along a fixed axis using a motorized shaft **1405**. The fixed axis can be vertical. In the embodiment shown in FIG. 27, a geared arrangement **1406** enables the motor **1601** to be placed perpendicular to the shaft **1405**, thereby saving space in the apparatus in which magnetic separator **1400** is situated. In other embodiments, the motor is placed underneath shaft **1405**. It would be understood that other configurations are consistent with the movement of the magnet relative to the process tubes, including, but not limited to, moving the magnet from side-to-side, or bringing the magnet down from above. The motor can be computer controlled to run at a particular speed; for example at a rotational speed that leads to vertical motion of the magnet in the range 1-20 mm/s. The magnetic separator can thus be configured to move repetitively, e.g., up and down, from side to side, or backwards and forwards, along the same axis several times. In some embodiments there is more than one shaft that operates under motorized control. The presence of at least a second shaft has the effect of making the motion of the separator more smooth. In some embodiments, the supporting member rides on one more guiding members to ensure that the supporting member does not, for example, tip, twist, or yaw, or undergo other internal motions while moving (other than that of controlled motion along the axis) and thereby reduce efficacy of the separation.

The supporting member can also be configured to move the magnets between a first position, situated away from the one or more receptacles, and a second position situated in close proximity to the one or more receptacles, and is further configured to move at an amplitude about the second position where the amplitude is smaller than a distance between the first position and the second position as measured along the shaft.

Shown in FIGS. 26 and 27, the heater assembly **1401** and the magnetic separator **1400** can be controlled by electronic circuitry such as on printed circuit board **1409**. The electronic circuitry **1409** can be configured to cause the heater assembly **1401** to apply heat independently to the process tubes **1402** to minimize the cost of heating and sensing. It can also be configured to cause the magnetic separator **1400** to move repetitively relative to the process tubes **1402**. The electronic circuitry **1409** can be integrated into a single printed circuit board (PCB). During assembly, a plastic guide piece can help maintain certain spacing between individual heat blocks **1403**. This design can benefit from use off-the-shelf electronics to control a custom arrangement of heat blocks **1403**.

Not shown in FIGS. 26 and 27, an enclosure can cover the magnetic separator **1400** and the heater assembly **1401** for protection of sub-assemblies below and aesthetics. The enclosure can also be designed to keep the heat blocks **1403** spaced apart from one another to ensure efficiency of heating and cooling. The magnetic separator and heater assembly can, alternatively, be enclosed by separate enclosures. The one or more enclosures can be made of plastic.

Advantageously, the heater assembly and magnetic separator operate together to permit successive heating and separation operations to be performed on liquid materials in the one or more process tubes without transporting either the liquid materials or the process tubes to different locations to perform either heating or separation. Such operation is also advantageous because it means that the functions of heating and separation which, although independent of one another, are both utilized in sample preparation may be performed with a compact and efficient apparatus.

Cartridge Autoloader

An exemplary embodiment of a PCR amplification-detection system **2900** for use with a microfluidic cartridge is shown in FIG. **28**. The system **2900** performs and automates the process of PCR on multiple nucleic-acid containing samples in parallel. The system **2900** comprises a depository **2907** for unused microfluidic cartridges, a cartridge auto-loader, a receiving bay for a microfluidic cartridge, a detector, and a waste tray **2903** configured to receive used microfluidic cartridges. In one embodiment, the cartridge autoloader comprises a cartridge pack **2901**, and a cartridge pusher **2904**.

The system **2900**, for illustration purposes, is configured so that a microfluidic cartridge moves in a plane and in a linear manner from the depository to the receiving bay, to the waste bin, but it need not be so arranged. For example, the waste cartridge bin **2903** can be aligned orthogonally, or any angle thereof, to the receiving bay, such as disposed behind it. Alternatively, each element (cartridge autoloader **2901**, receiving bay **2902**, and waste cartridge bin **2903**) can be configured in a step-wise manner where the cartridge pack **2901** is on the same, higher or lower level than the microfluidic PCR amplification-detection system **2902** and the microfluidic PCR amplification-detection system **2902** is on the same, higher or lower level than the waste cartridge bin **2903**. Another configuration could be that each of the three elements is not arranged linearly but at an angle to one another, although within the same plane.

FIG. **28** illustrates the cartridge pack **2901** and the waste cartridge bin **2903** below the plane of the receiving bay, and a detection system **2908** above the plane. This configuration is exemplary and it would be understood that these elements may be positioned above or below the plane in other embodiments.

FIG. **29** illustrates a depository for unused microfluidic cartridges. The depository can be configured to accept a number of individually stacked and individually loaded cartridges, or can be configured to accept a pack of cartridges. An exemplary cartridge pack has 24 cartridges. The depository may consist of a cage **2910** of any material that may or may not be transparent. For example it may be made of metal or plastic. The cartridge pack **2901** is not limited to twenty-four cartridges **106** per pack but may contain any number from 2 to 100. For example, other numbers such as 2, 4, 8, 10, 12, 16, 20, 30, 36, 40, 48, 50, or 64 are possible numbers of cartridges **106** per pack. Similarly, the depository may be configured to accept those numbers of cartridges, when individually stacked. In one embodiment, as in FIG. **29**, each cartridge **2906**, individually stacked, rests on ledges **2911** that protrude from the cage **2910**. However, other configurations are possible. For example, a cartridge **2906** may rest on recessed grooves made within the interior surfaces of cage **2910**. Furthermore, the cartridge pack **2901** may not need to be placed in a cage **2910**. The cartridge pack **2901** may itself include the necessary connections to bind securely to the apparatus to load the cartridges **2906**.

FIG. **30** is an illustration of an exemplary initial loading position of a cartridge pack **2901** in a depository when samples are loaded in the topmost cartridge in the pack. FIG. **30** shows the cartridge pack **2901** below a plane that contains a cartridge pusher. In other embodiments, the cartridge pack **2901** may be above the plane of a cartridge pusher where the pusher pushes the lowest cartridge out from the holder; or partly above and partly below in a holder **2920** where a cartridge pusher pushes a cartridge from the middle of the cartridge pack **2901**. In the embodiment shown, a topmost cartridge **106** is pushed along two guide

rails **2905**. Alternatively, there may be more or fewer guide rails (such as one or three) or no guide rails at all so long as a cartridge **2906** can be caused to move to other required positions.

An exemplary cartridge pusher **2904** is shown in FIG. **31**. The cartridge pusher **2904** pushes a cartridge **2906** along guide rails **2905**, which allows a cartridge **2906** to travel to pre-calibrated positions by the mechanism of a stepper motor **2930**. However, it would be understood that the mechanism of transporting the cartridge **2906** is not limited to a stepper motor **2930** and thus other mechanisms are also consistent with the cartridge pusher **2904** as described herein.

FIG. **32** shows a used cartridge **2906** that has been pushed by the cartridge pusher **2904** into the waste cartridge bin **2903** after a PCR process has been completed. The embodiment shows a lipped handle **2940** that facilitates easy handling, such as emptying, of the bin **2903**. However, it would be understood that the handle **2904** is not limited to the style and shape shown.

An exemplary cartridge pack **2901**, before and after multiple PCR processes are completed are shown in FIG. **33**. After the cartridge pusher **2904** pushes a cartridge **2906** out of the cartridge pack **2901**, a spring **2950** at the bottom of the cartridge pack pushes against the lower surface of the stack of cartridges and causes the topmost cartridge to be made available for sample injection. The spring **2950** is not limited in number or type. Thus although a single helical or coiled spring is shown, it is consistent with the description herein that more than one helical or coiled springs could be used, such as 2, 3, or 4, and that alternatively a sprung metal strip, or several strips, could be used. Alternatively another mechanism for forcing the cartridges upwards could be deployed, such as a pneumatic, hydraulic, or inflatable pressurized container, could be utilized.

It is to be noted that microfluidic cartridges, as further described herein, that have a raised lip along their edges to permit ease of stacking and/or storage in a pack or an auto-loader are particularly advantageous because the raised lips also introduce a stiffness into the cartridges and assist in keeping the fluid inlets on one cartridge away from those on another cartridge during storage and transport. The raised regions, which need not only be lips along each edge of a cartridge, also help minimize friction between the lower surface of one cartridge and the upper surface of another.

Cartridge Receiving Bay

The present technology relates to an apparatus and related methods for amplifying, and carrying out diagnostic analyses on, nucleotides from biological samples. The apparatus is configured to act on a disposable microfluidic cartridge containing multiple sample lanes in parallel, and comprises a reusable instrument platform that can actuate on-cartridge operations, can detect and analyze the products of the PCR amplification in each of the lanes separately, in all simultaneously, or in groups simultaneously, and, optionally, can display the results on a graphical user interface.

FIG. **34** shows a perspective view of an exemplary cartridge **200** that contains multiple sample lanes, and exemplary read head **300** that contains detection apparatus for reading signals from cartridge **200**. Also shown in FIG. **34** is a tray **110** that, optionally, can accommodate cartridge **200** prior to insertion of the cartridge in a receiving bay. The apparatus described herein is able to carry out real-time PCR on a number of samples in cartridge **200** simultaneously. Preferably the number of samples is 12 samples, as illustrated with exemplary cartridge **200**, though other numbers of samples such as 4, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40,

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and 48 are within the scope of the present description. In preferred operation of the apparatus, a PCR-ready solution containing the sample, and, optionally, one or more analyte-specific reagents (ASR's) using other components of the apparatus, as further described herein, prior to introduction into cartridge 200.

In some embodiments, an apparatus includes a bay configured to selectively receive a microfluidic cartridge; at least one heat source thermally coupled to the bay; and coupled to a processor as further described herein, wherein the heat source is configured to heat individual sample lanes in the cartridge, and the processor is configured to control application of heat to the individual sample lanes, separately, in all simultaneously, or in groups simultaneously.

In some embodiments, an apparatus further includes at least one detector configured to detect a polynucleotide (nucleic acid) in a sample in one or more of the individual sample lanes, separately or simultaneously; wherein the processor is coupled to the detector to control the detector and to receive signals from the detector.

The bay can be a portion of the apparatus that is configured to selectively receive the microfluidic cartridge. For example, the bay and the microfluidic cartridge can be complementary in shape so that the microfluidic cartridge is selectively received in, e.g., a single orientation. For example, the microfluidic cartridge can have a registration member that fits into a complementary feature of the bay. The registration member can be, for example, a cut-out on an edge of the cartridge, such as a corner that is cut-off, or one or more notches that are made on one or more of the sides. By selectively receiving the cartridge, the bay can help a user to place the cartridge so that the apparatus can properly operate on the cartridge. In this way, error-free alignment of cartridges can be achieved. Moreover, the cartridge can be designed to be slightly smaller than the receiving bay by approximately 200-300 micron for easy placement and removal of the cartridge. The apparatus can further include a sensor configured to sense whether the microfluidic cartridge is selectively received.

The bay can also be configured so that various components of the apparatus that can operate on the microfluidic cartridge (heat sources, detectors, force members, and the like) are positioned to properly operate on the microfluidic cartridge. For example, a contact heat source can be positioned in the bay such that it can be thermally coupled to a distinct location at a microfluidic cartridge that is selectively received in the receiving bay.

Alternatively, in connection with alignment of microheaters in the heater module with corresponding heat-requiring microcomponents (such as valves, pumps, gates, reaction chambers, etc), the microheaters can be designed to be slightly bigger than the heat requiring microfluidic components so that even though the cartridge may be off-centered from the heater, the individual components can still function effectively.

The detector 300 can be, for example, an optical detector, as further described herein. For example, the detector can include a light source that selectively emits light in an absorption band of a fluorescent dye, and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. Alternatively, for example, the optical detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye; or for example, the optical

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detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof; or for example, the optical detector can be configured to independently detect a plurality of fluorescent dyes at a plurality of different locations on a microfluidic cartridge, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof in a different sample.

The heat source can be, for example, a heat source such as a resistive heater or network of resistive heaters, a reversible heat source such as a liquid-filled heat transfer circuit or a thermoelectric element, a radiative heat source such as a xenon lamp, and the like.

In preferred embodiments, the at least one heat source can be a contact heat source selected from a resistive heater (or network thereof), a radiator, a fluidic heat exchanger and a Peltier device. The contact heat source can be configured at the receiving bay to be thermally coupled to one or more distinct locations of a microfluidic cartridge received in the bay, whereby the distinct locations are selectively heated. At least one additional contact heat source can be included, wherein the contact heat sources are each configured at the bay to be independently thermally coupled to a different distinct location in a microfluidic cartridge received in the bay, whereby the distinct locations are independently heated. The contact heat source can be configured to be in direct physical contact with a distinct location of a microfluidic cartridge received in the bay. In various embodiments, each contact source heater can be configured to heat a distinct location having an average diameter in 2 dimensions from about 1 millimeter (mm) to about 15 mm (typically about 1 mm to about 10 mm), or a distinct location having a surface area of between about 1 mm² about 225 mm² (typically between about 1 mm² and about 100 mm², or in some embodiments between about 5 mm² and about 50 mm²).

In various embodiments, at least one heat source can be a radiative heat source configured to direct heat to a distinct location of a microfluidic cartridge received in the receiving bay.

In various embodiments, the apparatus includes one or more force members that are configured to apply force to thermally couple the at least one heat source to at least a portion of the microfluidic cartridge received in the bay. The one or more force members can be configured to operate a mechanical member at the microfluidic cartridge. At least one force member can be manually operated. At least one force member can be mechanically coupled to a lid at the receiving bay, whereby operation of the lid operates the force member.

In various embodiments, the force applied by the one or more force members can result in an average pressure at an interface between a portion of the receiving bay and a portion of the microfluidic cartridge of about 1 psi. The application of force is important to ensure consistent thermal contact between the heater wafer and the PCR reactor and microvalves in the microfluidic cartridge.

In various embodiments, the apparatus can further include a lid at the receiving bay, the lid being operable to at least partially exclude ambient light from the bay. The lid can be, for example, a sliding lid. The lid can include the optical detector. A major face of the lid at the bay can vary from planarity by less than about 100 micrometers, for example, less than about 25 micrometers. The lid can be configured to be removable from the apparatus. The lid can include a

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latching member that ensures that the lid is securely closed before amplification reactions are applied to the samples in the cartridge.

FIG. 35 shows a schematic cross-sectional view of a part of an apparatus as described herein, showing input of sample into a cartridge 200 via a pipette tip 10 (such as a disposable pipette) attached to an automated dispensing head, and an inlet 202. Although not shown, there are as many inlets 202 as samples to be input into cartridge 200. Inlet 202 is preferably configured to receive a pipette or the bottom end of a PCR tube and thereby accept sample for analysis with minimum waste, and with minimum introduction of air. Cartridge 200 is disposed on top of and in contact with a heater substrate 400. Read head 300 is positioned above cartridge 200 and a cover for optics 310 restricts the amount of ambient light that can be detected by the read head.

In various embodiments, a system as described herein can include both a microfluidic cartridge and the diagnostic apparatus.

Microfluidic Cartridge

One aspect of the present technology relates to a microfluidic cartridge including a first, second, and third, layers that together define a plurality of microfluidic networks, each network having various components configured to carry out PCR on a sample having one or more polynucleotides whose presence is to be determined. The cartridge includes one or more sample lanes in parallel, wherein each lane is independently associated with a given sample for simultaneous processing, and each lane contains an independently configured microfluidic network. An exemplary cartridge having such a construction is shown in FIG. 36. Such a cartridge is simple to manufacture, and permits PCR in a concentrated reaction volume (~4 μ l) and enables rapid thermocycling, at ~20 seconds per cycle.

Although other layers may be found in cartridges having comparable performance and ease of manufacture, the cartridge herein includes embodiments having only three layers in their construction: a substrate having an upper side and an opposed lower side, wherein the substrate comprises a microfluidic network having a plurality of sample lanes; a laminate attached to the lower side to seal the components of the microfluidic network, and provide an effective thermal transfer layer between a dedicated heating element and components in the microfluidic network; and a label, attached to the upper side that also covers and seals holes that are used in the manufacturing process to load microfluidic components such as valves. Thus, embodiments herein include microfluidic cartridges consisting of three layers, a substrate, a laminate, and a label, though other, additional, features other than layers may be consistent with such characterizations. Embodiments herein further include microfluidic cartridges consisting essentially of three layers, a substrate, a laminate, and a label, though other, additional, features other than layers may be consistent with such characterizations. Furthermore, embodiments herein still further include microfluidic cartridges comprising three layers, a substrate, a laminate, and a label.

A microfluidic network can include, in fluidic communication, one or more components selected from the group consisting of: gates, valves such as thermally actuated valves, channels, vents, and reaction chambers. Particular components of exemplary microfluidic networks are further described elsewhere herein. The cartridge typically processes the sample by increasing the concentration of a polynucleotide to be determined.

A sample lane is a set of elements, controllable independently of those in another sample lane, by which a sample

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can be accepted and analyzed, according to methods described herein. A lane comprises at least a sample inlet, and a microfluidic component, as further described herein in connection with a microfluidic cartridge. In some embodiments, each microfluidic network additionally comprises an overflow reservoir to contain extra liquid dispensed into the cartridge.

In various embodiments, a lane can include a sample inlet port, a first thermally actuated valve, a second thermally actuated valve, a PCR reaction chamber, and channels connecting the inlet port to the PCR reaction chamber via the first valve, and channels connecting the PCR reaction chamber to an exit vent via the second valve. The sample inlet valve can be configured to accept a quantity of sample at a pressure differential compared to ambient pressure of between about 100 to 5000 Pa. It should be noted that the lower the loading pressure, the higher the fill time for a aliquot of reaction mix to fill the microfluidic network. Applying more pressure will reduce the fill time, but if the time for which the pressure is applied is not determined correctly, the sample could be blown out through the microfluidic cartridge (if an end hydrophobic vent is not present). Therefore the time for which the pressure is applied should be properly determined, such as by methods available to one of ordinary skill in the art, to prevent underfill or overfill. In general, the fill time is inversely proportional to the viscosity of the solution. For example, FIG. 37 shows a microfluidic cartridge containing twelve independent sample lanes capable of independent (simultaneous or successive) processing of samples.

The microfluidic network in each lane is typically configured to carry out PCR on a PCR-ready sample, such as one containing nucleic acid (DNA or RNA) extracted from a raw biological sample using other aspects of the apparatus as further described herein. A PCR-ready sample is thus typically a mixture comprising the PCR reagent(s) and the neutralized polynucleotide sample, suitable for subjecting to thermal cycling conditions that create PCR amplicons from the neutralized polynucleotide sample. For example, a PCR-ready sample can include a PCR reagent mixture comprising a polymerase enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid and a plurality of nucleotides, and at least one probe that is selective for a polynucleotide sequence.

Typically, the microfluidic network is configured so that the time required for a microdroplet of sample to pass from the inlet to the second valve is less than 50% of the time required for the sample to travel up to the exit vent. Typically, the microfluidic network is designed to have an increased flow resistance downstream of the two valves without increasing the total volume of the microfluidic network in comparison to the amount required to fill from the first valve to the end vent of the network.

FIG. 38A shows a perspective view of a portion of an exemplary microfluidic cartridge 200 according to the present technology. The cartridge may be referred to as a multi-lane PCR cartridge with dedicated pipette inlets 202. Shown in FIG. 38A are various representative components of cartridge 200. For example, sample inlet 202 is configured to accept a syringe, a pipette, or a PCR tube containing a PCR ready sample. More than one inlet 202 is shown, wherein one inlet operates in conjunction with a single lane. Various components of microfluidic circuitry in each lane are also visible. For example, microvalves 204, and 206, and vents 208, are parts of microfluidic circuitry in a given lane. Also shown is an ultrafast PCR reactor 210, which, as further described herein, is a microfluidic channel that is

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long enough to permit PCR to occur in a sample. Above PCR reactor **210** is a window **212** that permits optical detection, such as detection of fluorescence from a fluorescent substance, such as a fluorogenic hybridization probe, in PCR reactor **210** when a detector is situated above window **212**.

A multi-lane cartridge is configured to accept a number of samples, in particular embodiments 12 samples, wherein the samples include at least a first sample and a second sample, wherein the first sample and the second sample each contain one or more polynucleotides in a form suitable for amplification. The polynucleotides in question may be the same as, or different from one another, in different lanes of a cartridge. The multi-sample cartridge comprises at least a first microfluidic network and a second microfluidic network, adjacent to one another, wherein each of the first microfluidic network and the second microfluidic network is as elsewhere described herein, and wherein the first microfluidic network accepts the first sample, and wherein the second microfluidic network accepts the second sample.

The sample inlets of adjacent lanes are reasonably spaced apart from one another to prevent any contamination of one sample inlet from another sample when a user introduces a sample into any one cartridge. In some embodiments, the sample inlets are configured so as to prevent subsequent inadvertent introduction of sample into a given lane after a sample has already been introduced into that lane.

In some embodiments, the multi-sample cartridge has a size substantially the same as that of a 96-well plate as is customarily used in the art. Advantageously, then, the cartridge may be used with plate handlers used elsewhere in the art. Still more preferably, however, the multi-sample cartridge is designed so that a spacing between the centroids of sample inlets is 9 mm, which is an industry-recognized standard. This means that, in certain embodiments the center-to-center distance between inlet holes in the cartridge that accept samples from PCR tubes, as further described herein, is 9 mm. The inlet holes are manufactured frustoconical in shape with an appropriate conical angle so that industry-standard pipette tips (2 μ l, 20 μ l, 200 μ l, volumes, etc.) fit snugly, entering from the widest point of the inlet. Thus, in certain embodiments, an inlet comprises an inverted frustoconical structure of at least 1 mm height, and having a diameter at its widest point that accepts entry of a pipette tip, of from 1-5 mm. The apparatus herein may be adapted to suit other, later-arising, industry standards for pipette tips not otherwise described herein. Typically the volume of sample accepted via an inlet into a microfluidic network in a sample lane is from 1-20 μ l, and may be from 3-5 μ l. The inlet hole can be designed to fit a pipette tip snugly and to create a good seal around the pipette tip, within the cone of the inlet hole. However, the cone is designed such that the sealing is reversible because it is undesirable if the seal is so tight that the cartridge can be pulled away from its tray, or location in the receiving bay, when the pipette tips are lifted after the dispensing operations.

FIG. 37 shows a plan view of an exemplary microfluidic cartridge having 12 lanes. The inlet ports have a 6 mm spacing, so that, when used in conjunction with an automated sample loader having 4 heads, spaced equidistantly at 9 mm apart, the inlets can be loaded in three batches of 4 inlets: e.g., inlets 1, 4, 7, and 10 together, followed by 2, 5, 8, and 11, then finally 3, 6, 9, and 12, wherein the 12 inlets are numbered consecutively from one side of the cartridge to the other.

FIG. 39A shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIGS. 38A and 38B. FIG. 39B shows

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another plan view (left panel) of another representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIG. 36, and shows how the circuit is visible through the cartridge construction (right panel). Other configurations of microfluidic network would be consistent with the function of the cartridges and apparatus described herein. In sequence, sample is introduced through liquid inlet **202**, and optionally flows into a bubble removal vent channel **208** (which permits adventitious air bubbles introduced into the sample during entry, to escape), and continues along a channel **216**. Typically, when using a robotic dispenser of liquid sample, the volume is dispensed accurately enough that formation of bubbles is not a significant problem, and the presence of vent channel **208** is not necessary.

Throughout the operation of cartridge **200** the fluid is manipulated as a microdroplet (not shown in FIGS. 39A,B). Valves **204** and **206** are shown in FIG. 39A as double-valves, having a source of thermally responsive material (also referred to as a temperature responsive substance) on either side of the channel where they are situated. However, valves **204** and **206** may either or both be single valves that have a source of thermally responsive material on only one side of the respective channels. Valves **204** and **206** are initially open, so that a microdroplet of sample-containing fluid can be pumped into PCR reactor **210** from inlet hole **202**. Upon initiating of processing, the detector present on top of the PCR reactor checks for the presence of liquid in the PCR reactor, and then closes valves **204** and **206** to isolate the PCR reaction mix from the channels on either side.

The PCR reactor **210** is a microfluidic channel that is heated through a series of cycles to carry out amplification of nucleotides in the sample, as further described herein. Typically the PCR reactor has a volume of 3-5 μ l, in particular, 4 μ l. The inside walls of the channel in the PCR reactor are made very smooth and polished to a shiny finish (for example, using a polish selected from SPI A1, SPI A2, SPI A3, SPI b1, or SPI B2) during manufacture. This is in order to minimize any microscopic air trapping in the surface of the PCR reactor, which would cause bubbling during the thermocycling steps. The presence of bubbles especially in the detection region of the PCR reactor might cause a false reading for the PCR reaction. Furthermore, the PCR reactor **210** is made shallow such that the temperature gradient across the depth of the channel is minimized. The region of the cartridge **212** above PCR reactor **210** permits a detector to monitor progress of the reaction and also to detect fluorescence from a probe that binds to a quantity of amplified nucleotide. The region **212** is made of thinner material than the rest of the cartridge so as to permit the PCR reactor to be more responsive to a heating cycle (for example, to rapidly heat and cool between temperatures appropriate for denaturing and annealing steps), and so as to reduce glare, autofluorescence, and undue absorption of fluorescence. Both valves **204** and **206** are closed prior to thermocycling to prevent any evaporation of liquid, bubble generation, or movement of fluid from the PCR reactor.

End vent **214** prevents a user from introducing any excess amount of liquid into the microfluidic cartridge, as well as playing a role of containing any sample from spilling over to unintended parts of the cartridge. A user may input sample volumes as small as an amount to fill from the bubble removal vent to the middle of the PCR reactor, or up to valve **204** or beyond valve **204**. The use of microvalves prevents both loss of liquid or vapor thereby enabling even a partially filled reactor to successfully complete a PCR thermocycling reaction. The application of pressure (such as ~1 psi) to

contact the cartridge to the heater of the instrument assists in achieving better thermal contact between the heater and the heat-receivable parts of the cartridge, and also prevents the bottom laminate structure from expanding, as would happen if the PCR channel was partially filled with liquid and the entrapped air would be thermally expanded during thermo-

cycling. In various embodiments, the microfluidic network can optionally include at least one hydrophobic vent additional to the end vent.

After PCR has been carried out on a sample, and presence or absence of a polynucleotide of interest has been determined, it is preferred that the amplified sample remains on the cartridge and that the cartridge is either used again (if one or more lanes remain open), or disposed of. Should a user wish to run a post amplification analysis, such as gel electrophoresis, the user may pierce a hole through the laminate of the cartridge, and recover an amount—typically about 1.5 microliter—of PCR product. The user may also place the individual PCR lane on a special narrow heated plate, maintained at a temperature to melt the wax in the valve, and then aspirate the reacted sample from the inlet hole of that PCR lane.

In various embodiments, the microfluidic network can optionally include at least one reservoir configured to contain waste.

In various embodiments, the microfluidic cartridge can further include a label, such as a computer-readable or scannable label. For example, the label can be a bar code, a radio frequency tag, or one or more computer-readable, or optically scannable, characters. The label can be positioned such that it can be read by a sample identification verifier as further described herein.

In various embodiments, during transport and storage, the microfluidic cartridge can be further surrounded by a sealed pouch. The microfluidic cartridge can be sealed in the pouch with an inert gas. The microfluidic cartridge can be disposable.

Microfluidic cartridge 200 can be fabricated as desired. Typically, the microfluidic cartridge layer includes a layer of polypropylene or other plastic label with pressure sensitive adhesive (typically between about 50 and 150 microns thick)

second side (disposed toward the label). Typically, all of the microfluidic networks together, including the PCI reactors, the inlet holes and the valves for isolating the PCR reaction chambers, are defined in a single substrate. The substrate is made of a material that confers rigidity on the substrate and cartridge, and is impervious to air or liquid, so that entry or exit of air or liquid during operation of the cartridge is only possible through the inlet or the vent.

Channels of a microfluidic network in a lane of cartridge 200 typically have at least one sub-millimeter cross-sectional dimension. For example, channels of such a network may have a width and/or a depth of about 1 mm or less (e.g., about 750 microns or less, about 500 microns, or less, about 250 microns or less).

The cartridge can further include a heat sealable laminate layer 222 (typically between about 100 and about 125 microns thick) attached to the bottom surface of the microfluidic substrate using, for example, heat bonding, pressure bonding, or a combination thereof. The laminate layer 222 may also be made from a material that has an adhesive coating on one side only, that side being the side that contacts the underside of the microfluidic substrate. This layer may be made from a single coated tape having a layer of Adhesive 420, made by 3M. Exemplary tapes include single-sided variants of double sided tapes having product nos. 9783, 9795, and 97951, and available from 3M. Other acceptable layers may include tapes based on micro-capsule based adhesives.

In use, cartridge 200 is typically thermally associated with an array of heat sources configured to operate the components (e.g., valves, gates, and processing region 210) of the device. In some embodiments, the heat sources are operated by an operating system, which operates the device during use. The operating system includes a processor (e.g., a computer) configured to actuate the heat sources according to a desired protocol. Processors configured to operate microfluidic devices are described in, e.g., U.S. application Ser. No. 09/819,105, filed Mar. 28, 2001, which application is incorporated herein by reference.

Table 1 outlines volumes, pumping pressures, and operation times associated with various components of a microfluidic cartridge.

TABLE 1

Operation	Pumping Pressure	Displacement Volume	Time of Operation
Mixing displacements	~2 psi	10-25 μ l	1-2 minutes
Moving valve wax plugs	~1-2 psi	<1 μ l	5-15 seconds
Operation	Pump Used	Pump Design	Pump Actuation
Mixing displacements	Expancel Pump	Same as above	Same as above
Moving valve wax plugs	Thermopneumatic pump	1 μ l of trapped air	Heat trapped air to ~70-90 C.

configured to seal the wax loading holes of the valves, trap air used for valve actuation, and serve as a location for operator markings. This layer can be in two separate pieces, though it would be understood by one of ordinary skill in the art that in many embodiments a single piece layer would be appropriate.

The microfluidic substrate layer, is typically injection molded opt of a plastic, preferably a zeonor plastic (cyclic olefin polymer), having a PCR channel and valve channels on a first side, and vent channels and various inlet holes, including wax loading holes and liquid inlet holes, on a

In some embodiments, a microfluidic cartridge further comprises a registration member that ensures that the cartridge is received by a complementary diagnostic apparatus in a single orientation, for example, in a receiving bay of the apparatus. The registration member may be a simple cut-out from an edge or a corner of the cartridge (as shown in FIG. 38A), or may be a series of notches, or some other configuration of shapes that require a unique orientation of placement in the apparatus.

In some embodiments, the microfluidic cartridge comprises two or more positioning elements, or fiducials, for use

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when filling the valves with thermally responsive material. The positioning elements may be located on the substrate, typically the upper face thereof.

The microfluidic cartridges may also be stackable, such as for easy storage or transport, or may be configured to be received by a loading device, as further described herein, that holds a plurality of cartridges in close proximity to one another, but without being in contact. In order to accomplish either or both of these characteristics, the substrate may comprise two ridges, one of each situated along each of two opposite edges of the cartridge, the ridges disposed on the upper side of the substrate. Thus, where a cartridge has a rectangular aspect (ignoring any registration member or mechanical key), the two ridges may be situated along the long side, or along the short side, of the cartridge.

Valves

A valve is a microfluidic component that has a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). An exemplary double valve is shown in FIG. 40A. A double valve has two channels, one on either side of the channel whose flow it regulates, whereas a single valve has just one channel, disposed on one side of the channel whose flow it regulates.

Upon actuation, e.g., by application of heat, the valve transitions to a closed state that prevents material, such as a microdroplet of PCR-ready sample, from passing along the channel from one side of the valve to the other. For example, a valve includes one or more masses of a thermally responsive substance (TRS) that is relatively immobile at a first temperature and more mobile at a second temperature. A mass of TRS can be an essentially solid mass or an agglomeration of smaller particles that cooperate to obstruct the passage upon actuation. Examples of TRS's include a eutectic alloy (e.g., a solder), wax (e.g., an olefin), polymers, plastics, and combinations thereof. The first and second temperatures are insufficiently high to damage materials, such as polymer layers of a microfluidic cartridge in which the valve is situated. Generally, the second temperature is less than about 90° C. and the first temperature is less than the second temperature (e.g., about 70° C. or less).

For each mass associated with a valve, a chamber is in gaseous communication with the mass. Upon heating gas (e.g., air) in the chamber(s) and heating the one or more masses of TRS to the second temperature, gas pressure within a chamber moves the corresponding mass into the channel obstructing material from passing therealong. Other valves of the network have the same structure and operate in the same fashion as the valves described herein.

In order to make the valve sealing very robust and reliable, the flow channel at the valve junction is made narrow (150 μ m wide and 150 μ m deep or narrower) and the constricted channel is made at least 0.5 or 1 mm long such that the wax seals up a long narrow channel thereby reducing any leakage through the walls of the channel. In the case of a bad seal, there is leakage of fluid around the walls of the channel, past the wax. So the flow channel is narrowed as much as possible, and made longer, e.g., as long as ~1 mm. The valve operates by heating air in the wax-loading port, which forces the wax forwards in a manner so that it does not come back to its original position. In this way, both air and wax are heated during operation of the valve.

In various embodiments, the microfluidic network can include a bent valve as shown in FIG. 32B (as a single valve) to reduce the footprint of the valve on the cartridge and hence reduce cost per part for manufacturing highly dense

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microfluidic substrates. In the valve of FIG. 40B, the loading hole for TRS is in the center of the valve; the structures at either end are an inlet and an outlet and are shown for illustrative purposes only. Single valve shown.

In various embodiments, the network can include a curved valve as shown in FIG. 40C, also as a single valve, in order to reduce the effective cross-section of the micro-valve, enabling manufacture of cheaper dense microfluidic devices.

Vents

A hydrophobic vent (e.g., a vent in FIG. 41) is a structure that permits gas to exit a channel while limiting (e.g., preventing) liquid from exiting the channel. Typically, hydrophobic vents include a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane from Osmonics) that defines a wall of the channel. As discussed herein, hydrophobic vents can be used to position a microdroplet of sample at a desired location within a microfluidic network.

The hydrophobic vents of the cartridge are preferably constructed so that the amount of air that escapes through them is maximized while minimizing the volume of the channel below the vent surface. Accordingly, it is preferable that the vent is constructed so as to have a hydrophobic membrane of large surface area and a shallow cross section of the microchannel below the vent surface.

Bubble removal hydrophobic vents typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least about 7.5 mm) along a channel. The length of the hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydrophobic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less). Bubble vents are optional in the microfluidic networks of the microfluidic cartridges described herein.

The depth of the channel within the hydrophobic vent is typically about 75% or less (e.g., about 65% or less, about 60% or less) of than the depth of the channel upstream and downstream of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and downstream of the hydrophobic vent is about 250 microns.

A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50% wider) than a width of the channel upstream from the vent and downstream from the vent. For example, in an exemplary embodiment, the width of the channel within the hydrophobic vent is about 400 microns and the width of the channel upstream and downstream from the vent is about 250 microns.

Highly Multiplexed Embodiment

Embodiments of the apparatus and cartridge described herein may be constructed that have high-density microfluidic circuitry on a single cartridge that thereby permit processing of multiple samples in parallel, or in sequence, on a single cartridge. Preferred numbers of such multiple samples include 36, 40, 48, 50, 64, 72, 80, 96, and 100, but it would be understood that still other numbers are consistent with the apparatus and cartridge herein, where deemed convenient and practical.

Accordingly, different configurations of lanes, sample inlets, and associated heater networks are contemplated that can facilitate processing such numbers of samples on a single cartridge are within the scope of the instant disclosure.

sure. Similarly, alternative configurations of detectors for use in conjunction with such a highly multiplexed cartridge are also within the scope of the description herein.

In an exemplary embodiment, a highly multiplexed cartridge has 48 PCR channels, and has independent control of each valve in the channel, with 2 banks of thermocycling protocol per channel, as shown in FIG. 43. In the embodiment in FIG. 43, the heaters are arranged in three arrays. Heaters in two separate glass regions only apply heat to valves in the microfluidic networks in each lane. Because of the low thermal conductivity of glass, the individual valves may be heated separately from one another. This permits samples to be loaded into the cartridge at different times, and passed to the PCR reaction chambers independently of one another. The PCR heaters are mounted on a silicon substrate—and are not readily heated individually, but thereby permit batch processing of PCR samples, where multiple samples from different lanes are amplified by the same set of heating/cooling cycles. It is preferable for the PCR heaters to be arranged in 2 banks (the heater arrays on the left and right are not in electrical communication with one another), thereby permitting a separate degree of sample control.

FIG. 42 shows a representative cartridge, revealing an inlet configuration for a 48-sample cartridge. The inlet configuration is compatible with an automatic pipetting machine that has dispensing heads situated at a 9 mm spacing. For example, such a machine having 4 heads can load 4 inlets at once, in 12 discrete steps, for the cartridge of FIG. 42.

FIG. 44 shows, in close, up an exemplary spacing of valves and lanes in adjacent lanes of a multi-sample microfluidic cartridge.

FIGS. 45 and 46 show close-ups of, respectively, heater arrays, and inlets, of the exemplary cartridge shown in FIG. 44.

FIGS. 47A-47C show various views of an embodiment of a radially-configured highly-multiplexed cartridge, having a number of inlets, microfluidic lanes, and PCR reaction zones.

The various embodiments shown in FIGS. 42-47C are compatible with liquid dispensers, receiving bays, and detectors that are configured differently from the specific examples described herein.

In another preferred embodiment (not shown in the FIGs.), a cartridge and apparatus is configured so that the read-head does not cover the sample inlets, thereby permitting loading of separate samples while other samples are undergoing PCR thermocycling.

Heater Configurations to Ensure Uniform Heating of a Region

Another aspect of the apparatus described herein relates to a method and apparatus for uniformly controlling the heating of a region of a microfluidic network that includes but is not limited to one or more microfluidic components. In an exemplary embodiment, multiple heaters can be configured to simultaneously and uniformly heat a region, such as the PCR reaction zone, of the microfluidic cartridge.

In preferred embodiments, a microfluidic cartridge having a microfluidic network comprising one or more micro fluidic components is brought into contact with a heat source, within a suitably configured apparatus. The heat source is configured so that particular heating elements are situated to heat specific components of the microfluidic network of the cartridge.

FIG. 48 shows a cross-sectional view of an exemplary microfluidic cartridge to show relative location of PCR channel in relation to the heaters when the cartridge is placed

in the instrument. The view in FIG. 48 is also referred to as a sectional-isometric view of the cartridge lying over the heater wafer. A window 903 above the PCR channel in the cartridge is shown in perspective view. PCR channel 901 (for example, 150 μ deep \times 700 μ wide), is shown in an upper layer of the cartridge. A laminate layer 905 of the cartridge (for example, 125 μ thick) is directly under the PCR channel 901. A further layer of thermal interface laminate 907 on the cartridge (for example, 125 μ thick) lies directly under the laminate layer 905. Heaters are situated in a further layer 913 directly under the thermal interface laminate. The heaters are photolithographically defined and etched metal layers of gold (typically about 3,000 Å thick). Layers of 400 Å of TiW are deposited on top and bottom of the gold layer to serve as an adhesion layer. The substrate used is glass, fused silica or quartz wafer having a thickness of 0.4 mm, 0.5 mm or 0.7 mm or 1 mm. A thin electrically-insulative layer of 2 μ m silicon oxide serves as an insulative layer on top of the metal layer. Additional thin electrically insulative layers such as 2-4 μ m of Parylene may also be deposited on top of the Silicon oxide surface. Two long heaters 909 and 911, as further described herein, are also shown.

Referring to FIGS. 49A and 49B, the PCR reaction zone 1001, typically having a volume \sim 1.6 μ l, is configured with a long side and a short side, each with an associated heating element. The apparatus therefore preferably includes four heaters disposed along the sides of, and configured to heat, the PCR reaction zone, as shown in the exemplary embodiment of FIG. 38A: long top heater 1005, long bottom heater 1003, short left heater 1007, and short right heater 1009. The small gap between long top heater 1005 and long bottom heater 1003 results in a negligible temperature gradient (less than 1° C. across the width of the PCR channel at any point along the length of the PCR reaction zone) and therefore an effectively uniform temperature throughout the PCR reaction zone. The heaters on the short edges of the PCR reactor provide heat to counteract the gradient created by the two long heaters from the center of the reactor to the edge of the reactor. It would be understood by one of ordinary skill in the art that still other configurations of one or more heater(s) situated about a PCR reaction zone are consistent with the methods and apparatus described herein. For example, a 'long' side of the reaction zone can be configured to be heated by two or more heaters. Specific orientations and configurations of heaters are used to create uniform zones of heating even on substrates having poor thermal conductivity because the poor thermal conductivity of glass, or quartz, or fused silica substrates is utilized to help in the independent operation of various microfluidic components such as valves and independent operation of the various PCR lanes.

In preferred embodiments, each heater has an associated temperature sensor. In the embodiment of FIG. 49A, a single temperature sensor 1011 is used for both long heaters. A temperature sensor 1013 for short left heater, and a temperature sensor 1015 for short right heater are also shown. The temperature sensor in the middle of the reactor is used to provide feedback and control the amount of power supplied to the two long heaters, whereas each of the short heaters has a dedicated temperature sensor placed adjacent to it in order to control it. As further described herein, temperature sensors are preferably configured to transmit information about temperature in their vicinity to the processor at such times as the heaters are not receiving current that causes them to heat. This can be achieved with appropriate control of current cycles.

In order to reduce the number of sensor or heater elements required to control a PCR heater, we may use the heaters to

sense as well as heat, and thereby obviate the need to have a separate dedicated sensor for each heater. In another embodiment, each of the four heaters may be designed to have an appropriate wattage, and connect the four heaters in series or in parallel to reduce the number of electronically-controllable elements from 4 to just 1, thereby reducing the burden on the electronics.

FIG. 49B shows expanded views of heaters and temperature sensors used in conjunction with a PCR reaction zone of FIG. 49A. Temperature sensors **1001** and **1013** are designed to have a room temperature resistance of approximately 200-300 ohms. This value of resistance is determined by controlling the thickness of the metal layer deposited (e.g., a sandwich of 400 Å TiW/3000 Å Au/400 Å TiW), and etching the winding metal line to have a width of approximately 10-25 μm and 20-40 mm length. The use of metal in this layer gives it a temperature coefficient of resistivity of the order of 0.5-20° C./ohms, preferably in the range of 1.5-3° C./ohms. Measuring the resistance at higher temperatures will enable determination of the exact temperature of the location of these sensors.

The configuration for uniform heating, shown in FIG. 49A for a single PCR reaction zone, can be applied to a multi-lane PCR cartridge in which multiple independent PCR reactions occur.

Each heater can be independently controlled by a processor and/or control circuitry used in conjunction with the apparatus described herein. FIG. 50 shows thermal images, from the top surface of a microfluidic cartridge having heaters configured as in FIGS. 49A and 49B, when each heater in turn is activated, as follows: (A): Long Top only; (B) Long Bottom only; (C) Short Left only; (D) Short Right only; and (E) All Four Heaters on. Panel (F) shows a view of the reaction zone and heaters on the same scale as the other image panels in FIG. 50. Also shown in the figure is a temperature bar.

Use of Cutaways in Cartridge Substrate to Improve Rate of Cooling During PCR Cycling

During a PCR amplification of a nucleotide sample, a number of thermal cycles are carried out. For improved efficiency, the cooling between each application of heat is preferably as rapid as possible. Improved rate of cooling can be achieved with various modifications to the heating substrate, as shown in FIGS. 51A-51C.

One way to achieve rapid cooling is to cutaway portions of the microfluidic cartridge substrate, as shown in FIG. 51A. The upper panel of FIG. 51A is a cross-section of an exemplary microfluidic cartridge taken along the dashed line A-A' as marked on the lower panel of FIG. 51A. PCR reaction zone **901**, and representative heaters **1003** are shown. Also shown are two cutaway portions, one of which labeled **1201**, that are situated alongside the heaters that are situated along the long side of the PCR reaction zone. Cutaway portions such as **1201** reduce the thermal mass of the cartridge, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction zone. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology.

Another way to achieve rapid cooling is to cutaway portions of the heater substrate, as shown in FIG. 51B. The lower panel of FIG. 51B is a cross-section of an exemplary microfluidic cartridge and heater substrate taken along the dashed line A-A' as marked on the upper panel of FIG. 51B. PCR reaction zone **901**, and representative heaters **1003** are shown. Also shown are four cutaway portions, one of which

labeled **1205**, that are situated alongside the heaters that are situated along the long side of the PCR reaction zone. Cutaway portions such as **1205** reduce the thermal mass of the heater substrate, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction zone. Four separate cutaway portions are shown in FIG. 51B so that control circuitry to the various heaters is not disrupted. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology. These cutouts may be created by a method selected from: selective etching using wet etching processes, deep reactive ion etching, selective etching using CO₂ laser or femtosecond laser (to prevent surface cracks or stress near the surface), selective mechanical drilling, selective ultrasonic drilling, or selective abrasive particle blasting. Care has to be taken to maintain mechanically integrity of the heater while reducing as much material as possible.

FIG. 51C shows a combination of cutouts and use of ambient air cooling to increase the cooling rate during the cooling stage of thermocycling. A substantial amount of cooling happens by convective loss from the bottom surface of the heater surface to ambient air. The driving force for this convective loss is the differential in temperatures between the glass surface and the air temperature. By decreasing the ambient air temperature by use of, for example, a peltier cooler, the rate of cooling can be increased. The convective heat loss may also be increased by keeping the air at a velocity higher than zero.

An example of thermal cycling performance obtained with a configuration as described herein, is shown in FIG. 52 for a protocol that is set to heat up to 92° C., and stay there for 1 second, then cool to 62° C., and stay for 10 seconds. Cycle time is about 29 seconds, with 8 seconds required to heat from 62° C. and stabilize at 92° C., and 10 seconds required to cool from 92° C., and stabilize at 62° C.

Manufacturing Process for Cartridge

FIG. 53 shows a flow-chart **2800** for an assembly process for an exemplary cartridge as further described herein. It would be understood by one of ordinary skill in the art, both that various steps may be performed in a different order from that set forth in FIG. 53, and additionally that any given step may be carried out by alternative methods to those set forth in the figure. It would also be understood that, where separate steps are illustrated for carrying out two or more functions, such functions may be performed synchronously and combined into single steps and be consistent with the overall process described herein.

At **2802**, a laminate layer is applied to a microfluidic substrate that has previously been engineered to have a microfluidic network constructed in it; edges are trimmed from the laminate where they spill over the bounds of the substrate.

At **2804**, wax is dispensed and loaded into the microvalves of the microfluidic network in the microfluidic substrate. An exemplary process for carrying this out is further described herein.

At **2806**, the cartridge is inspected to ensure that wax from step **2804** is loaded properly and that the laminate from step **2802** adheres properly to the microfluidic substrate. If a substrate does not satisfy either or both of these tests, it is discarded. If substrates repeatedly fail either or both of these tests, then the wax dispensing, or laminate application steps, as applicable, are reviewed.

At **2808**, a hydrophobic vent membrane is applied to, and heat bonded to, the top of the microfluidic substrate over the wax valves, and on the opposite face of the substrate from

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the laminate. Edges of the membrane that are in excess of the boundary of the substrate are trimmed.

At **2810**, the assembly is inspected to ensure that the hydrophobic vent membrane is bonded well to the microfluidic substrate without heat-clogging the microfluidic channels. If any of the channels is blocked, or if the bond between the membrane and the substrate is imperfect, the assembly is discarded, and, in the case of repeated discard events, the foregoing process step is reviewed.

At **2812**, a thermally conductive pad layer is applied to the bottom laminate of the cartridge.

At **2814**, two label strips are applied to the top of the microfluidic substrate, one to cover the valves, and a second to protect the vent membranes. It would be understood that a single label strip may be devised to fulfill both of these roles.

At **2816**, additional labels are printed or applied to show identifying characteristics, such as a barcode #, lot # and expiry date on the cartridge. Preferably one or more of these labels has a space and a writable surface that permits a user to make an identifying annotation on the label, by hand.

At **2818**, to facilitate transport and delivery to a customer, assembled and labeled cartridges are stacked and pack cartridges in groups, such as groups of 25, or groups of 10, or groups of 20, or groups of 50. Preferably the packaging is via an inert and/or moisture-free medium.

Exemplary Wax-Deposition Process

Deposition of wax in valves of the microfluidic network, as at step **2804** may be carried out with the exemplary equipment shown in FIGS. **54A** and **54B**. The DispenseJet Series DJ-9000 (FIGS. **54A** and **54B**) is a non-contact dispenser that provides high-speed delivery and exceptional volumetric control for various fluids, including surface mount adhesive, underfill, encapsulants, conformal coating, UV adhesives, and silver epoxy. The DJ-9000 jets in tight spaces as small as 200 micrometers and creates fillet wet-out widths as small as 300 micrometers on the dispensed side of a substrate such as a die. It dispenses fluid either as discrete dots or a rapid succession of dots to form a 100-micron (4 mil) diameter stream of fluid from the nozzle. It is fully compatible with other commercially available systems such as the Asymtek Century C-718/C-720, Millennium M-2000, and Axiom X-1000 Series Dispensing Systems.

A DJ-9000 is manufactured by Asymtek under manufacturing quality control standards aim to provide precise and reliable performance. Representative specifications of the apparatus are as follows.

Characteristic	Specification
Size	Width: 35 mm Height: 110 mm Depth: 100 mm
Weight	400 grams - dry
Feed Tube Assembly	Nylon - Fitting Polyurethane - Tube
Fluid Chamber	Type 303 Stainless Steel
Seat and Nozzle	300/400 Series S/S, Carbide
Needle Assembly	52100 Bearing Steel - Shaft Hard Chrome Plate Carbide - Tip
Fluid Seal	PEEK/Stainless Steel
Fluid Chamber O-Ring	Ethylene Propylene
Jet Body	6061-T6 Aluminum Nickel Plated
Needle Assembly Bearings	PEEK
Thermal Control Body	6061-T6 Aluminum Nickel Plated

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-continued

Characteristic	Specification
Reservoir Holder	Acetyl
Reservoir Size	5, 10, or 30 cc (0.17, 0.34, or 1.0 oz)
Feed Tube Assembly Fitting	Female Luer per ANSI/HIMA MD70.1-1983
Maximum Cycle Frequency	200 Hz.
Minimum Valve Air Pressure	5.5 bar (80 psi)
Operating Noise Level	70 db*
Solenoid	24 VDC, 12.7 Watts
Thermal Control Heater	24 VDC, 14.7 Watts, 40 ohms
Thermal Control RTD	100 ohm, platinum
Maximum Heater Set Point	80 C

*At Maximum Cycle Rate

An exploded view of this apparatus is shown in FIG. **54B**. Theory of Operation of DJ-9000

The DJ-9000 has a normally closed, air-actuated, spring-return mechanism, which uses momentum transfer principles to expel precise volumes of material. Pressurized air is regulated by a high-speed solenoid to retract a needle assembly from the seat. Fluid, fed into the fluid chamber, flows over the seat. When the air is exhausted, the needle travels rapidly to the closed position, displacing fluid through the seat and nozzle in the form of a droplet. Multiple droplets fired in succession can be used to form larger dispense volumes and lines when combined with the motion of a dispenser robot.

The equipment has various adjustable features: The following features affect performance of the DJ-9000 and are typically adjusted to fit specific process conditions.

Fluid Pressure should be set so that fluid fills to the seat, but should not be influential in pushing the fluid through the seat and nozzle. In general, higher fluid pressure results in a larger volume of material jetted.

The Stroke Adjustment controls the travel distance of the Needle Assembly. The control is turned counterclockwise to increase needle assembly travel, or turned clockwise to decrease travel. An increase of travel distance will often result in a larger volume of material jetted.

The Solenoid Valve controls the valve operation. When energized, it allows air in the jet air chamber to compress a spring and thereby raise the Needle Assembly. When de-energized, the air is released and the spring forces the piston down so that the needle tip contacts the seat.

The seat and nozzle geometry are typically the main factors controlling dispensed material volume. The seat and nozzle size are determined based on the application and fluid properties. Other parameters are adjusted in accordance with seat and nozzle choices. Available seat and nozzle sizes are listed in the table hereinbelow.

Thermal Control Assembly: Fluid temperature often influences fluid viscosity and flow characteristics. The DJ-9000 is equipped with a Thermal Control Assembly that assures a constant fluid temperature.

Dot and Line Parameters: In addition to the DJ-9000 hardware configuration and settings, Dot and Line Parameters are set in a software program (referred to as FmNT) to control the size and quality of dots and lines dispensed. Wax Loading in Valves

FIGS. **55A** and **55B** show how a combination of controlled hot drop dispensing into a heated microchannel device of the right dimensions and geometry is used to accurately load wax into a microchannel of a microfluidic cartridge to form a valve. The heated dispenser head can be accurately position over an inlet hole of the microchannel in the microfluidic device, and can dispense molten wax drops in volumes as small as 75 nanoliters with an accuracy of

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20%. The inlet hole of the microchannel device is dimensioned in such a way that the droplet of 75 nl can be accurately shot to the bottom of the inlet hole using, for example, compressed air, or in a manner similar to an inkjet printing method. The microchannel device is maintained at a temperature above the melting point of the wax thereby permitting the wax to stay in a molten state immediately after it is dispensed. After the drop falls to the bottom of the inlet hole, the molten wax is drawn into the narrow channel by capillary action. The volume of the narrow section is designed to be approximately equal to a maximum typical amount that is dispensed into the inlet hole.

Heater Multiplexing (Under Software Control)

Another aspect of the apparatus described herein, relates to a method for controlling the heat within the system and its components, as illustrated in FIG. 56. The method leads to a greater energy efficiency of the apparatus described herein, because not all heaters are heating at the same time, and a given heater is receiving current for only pan of the time.

Generally, the heating of microfluidic components, such as a PCR reaction zone, is controlled by passing currents through suitably configured microfabricated heaters. The heating can be further controlled by periodically turning the current on and off with varying pulse width modulation (PWM), wherein pulse width modulation refers to the on-time/off-time ratio for the current. The current can be supplied by connecting a microfabricated heater to a high voltage source (for example, 30V), which can be gated by the PWM signal. In some embodiments, the device includes 48 PWM signal generators. Operation of a PWM generator includes generating a signal with a chosen, programmable period (the end count) and granularity. For instance, the signal can be 4000 μ s (micro-seconds) with a granularity of 1 μ s, in which case the PWM generator can maintain a counter beginning at zero and advancing in increments of 1 μ s until it reaches 4000 μ s, when it returns to zero. Thus, the amount of heat produced can be adjusted by adjusting the end count. A high end count corresponds to a greater length of time during which the microfabricated heater receives current and therefore a greater amount of heat produced.

In various embodiments, the operation of a PWM generator can also include a programmable start count in addition to the aforementioned end count and granularity. In such embodiments, multiple PWM generators can produce signals that can be selectively non-overlapping (e.g., by multiplexing the on-time of the various heaters) such that the current capacity of the high voltage power is not exceeded. Multiple heaters can be controlled by different PWM signal generators with varying start and end counts. The heaters can be divided into banks, whereby a bank defines a group of heaters of the same start count. For example, 36 PWM generators can be grouped into six different banks, each corresponding to a certain portion of the PWM cycle (500 ms for this example). The end count for each PWM generator can be selectively programmed such that not more than six heaters will be on at any given time. A portion of a PWM cycle can be selected as dead time (count 3000 to 4000 for this example) during which no heating takes place and sensitive temperature sensing circuits can use this time to sense the temperature. The table below represents a PWM cycle for the foregoing example:

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	Start Count	End Count	Max End count
Bank 1			
5 PWM generator#1	0	150	500
PWM generator#2	0	220	500
...
PWM generator#6	0	376	500
Bank 2			
10 PWM generator#7	500	704	1000
PWM generator#8	500	676	1000
...
PWM generator#12	500	780	1000
Bank 3			
15 PWM generator#13	1000	1240	1500
PWM generator#14	1000	1101	1500
...
PWM generator#18	1000	1409	1500
Bank 4			
20 PWM generator#19	1500	1679	2000
PWM generator#20	1500	1989	2000
...
PWM generator#24	1500	1502	2000
Bank 5			
25 PWM generator#25	2000	2090	2500
PWM generator#26	2000	2499	2500
...
PWM generator#30	2000	2301	2500
Bank 6			
30 PWM generator#31	2500	2569	3000
PWM generator#32	2500	2790	3000
...
PWM generator#36	2500	2678	3000

Use of Detection System to Measure/Detect Fluid in PCR Chamber

The apparatus optionally has a very sensitive fluorescence detector that is able to collect fluorescence light from the PCR chamber 210 of a microfluidic cartridge. This detector is used to detect the presence of liquid in the chamber, a measurement that determines whether or not to carry out a PCR cycle. A background reading is taken prior to filling the chamber with liquid. Another reading is taken after microfluidic operations have been performed that should result in filling the PCR chamber with liquid. The presence of liquid alters the fluorescence reading from the chamber. A programmable threshold value is used to tune an algorithm programmed into the processor (for example, the second reading has to exceed the first reading by 20%). If the two readings do not differ beyond the programmed margin, the liquid is deemed to not have entered the chamber, and a PCR cycle is not initiated for that chamber. Instead, a warning is issued to a user.

Computer Program Product

In various embodiments, a computer program product for use with the apparatus herein includes computer readable instructions thereon for operating the apparatus.

In various embodiments, the computer program product can include one or more instructions to cause the system to: output an indicator of the placement of the microfluidic cartridge in the bay; read a sample label or a microfluidic cartridge label; output directions for a user to input a sample identifier; output directions for a user to load a sample transfer member with the PCR-ready sample; output directions for a user to introduce the PCR-ready sample into the microfluidic cartridge; output directions for a user to place the microfluidic cartridge in the receiving bay; output direc-

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tions for a user to close the lid to operate the force member; output directions for a user to pressurize the PCR-ready sample in the microfluidic cartridge by injecting the PCR-ready sample with a volume of air between about 0.5 ml. and about 5 mL; and output status information for sample progress from one or more lanes of the cartridge.

In various embodiments, the computer program product can include one or more instructions to cause the system to: heat the PCR ready-sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide; contact the neutralized polynucleotide sample or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; independently contact each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide; contact the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; output a determination of the presence of a polynucleotide sequence in the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; and/or output a determination of a contaminated result if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof.

In various embodiments, the computer program product can include one or more instructions to cause the system to automatically conduct one or more of the steps of the method.

In various embodiments, the microfluidic cartridge comprises two or more sample lanes, each including a sample inlet valve, a bubble removal vent, a thermally actuated pump, a thermally actuated valve, and a PCR reaction zone, wherein the computer readable instructions are configured to independently operate one or more components of each said lane in the system, independently of one another, and for causing a detector to measure fluorescence from the PCR reaction zones.

Sample

In various embodiments, the sample can include a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides. The PCR reagent mixture can be in the form of one or more lyophilized pellets and the steps by which the PCR-ready sample is prepared can involve contacting the PCR pellet with liquid to create a PCR reagent mixture solution. In yet another embodiment, each of the PCR lanes may have dried down or lyophilized ASR reagents preloaded such that the user only needs to input prepared polynucleotide sample into the PCR. In another embodiment, the PCR lanes may have only the application-specific probes and primers premeasured and preloaded, and the user inputs a sample mixed with the PCR reagents.

In various embodiments, the microfluidic network can be configured to couple heat from an external heat source to a sample mixture comprising PCR reagent and neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

In various embodiments, the PCR ready sample can further include a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid. In various embodiments, the PCR-ready sample further includes a sample buffer, and at least one probe that

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is selective for a polynucleotide sequence, e.g., the polynucleotide sequence that is characteristic of a pathogen selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the microfluidic cartridge can accommodate a negative control polynucleotide, wherein the microfluidic network can be configured to independently carry out PCR on each of a neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide. Each lane of a multi-lane cartridge as described herein can perform two reactions because of the presence of two fluorescence detection systems per lane. A variety of combinations of reactions can be performed in the cartridge, such as two sample reactions in one lane, a positive control and a negative control in two other lanes; or a sample reaction and an internal control in one lane and a negative control in a separate lane.

In various embodiments, the sample can include at least one probe that can be selective for a polynucleotide sequence, wherein the steps by which the PCR-ready sample is prepared involve contacting the neutralized polynucleotide sample or a PCR amplicon thereof with the probe. The probe can be a fluorogenic hybridization probe. The fluorogenic hybridization probe can include a polynucleotide sequence coupled to a fluorescent reporter dye and a fluorescence quencher dye. The PCR reagent mixture can further include a positive control plasmid and a plasmid fluorogenic hybridization probe selective for at least a portion of the plasmid and the microfluidic cartridge can be configured to allow independent optical detection of the fluorogenic hybridization probe and the plasmid fluorogenic hybridization probe.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism, for example any organism that employs deoxyribonucleic acid or ribonucleic acid polynucleotides. Thus, the probe can be selective for any organism. Suitable organisms include mammals (including humans), birds, reptiles, amphibians, fish, domesticated animals, wild animals, extinct organisms, bacteria, fungi, viruses, plants, and the like. The probe can also be selective for components of organisms that employ their own polynucleotides, for example mitochondria. In some embodiments, the probe is selective for microorganisms, for example, organisms used in food production (for example, yeasts employed in fermented products, molds or bacteria employed in cheeses, and the like) or pathogens (e.g., of humans, domesticated or wild mammals, domesticated or wild birds, and the like). In some embodiments, the probe is selective for organisms selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of *Staphylococcus* spp., e.g., *S. epidermidis*, *S. aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (e.g., α , β or γ -hemolytic, Group A, B, C, D or G) such as *S. pyogenes*, *S. agalactiae*; *E. faecalis*, *E. durans*, and *E. faecium* (formerly *S. faecalis*, *S. durans*, *S. faecium*); nonenterococcal group D streptococci, e.g., *S. bovis* and *S. equines*; Streptococci *viridans*, e.g., *S. mutans*, *S. sanguis*, *S. salivarius*, *S. mitior*, *A. milleri*, *S. constellatus*, *S. intermedius*, and *S. anginosus*; *S. iniae*; *S. pneumoniae*; *Neisseria*, e.g., *N. meningitidis*, *N. gonorrhoeae*, sapro-

phytic *Neisseria* sp; *Erysiplothrax*, e.g., *E. rhusiopathiae*; *Listeria* spp., e.g., *L. monocytogenes*, rarely *L. ivanovii* and *L. seeligeri*; *Bacillus*, e.g., *B. anthracis*, *B. cereus*, *B. subtilis*, *B. subtilis niger*, *B. thuringiensis*; *Nocardia* asteroids; *Legionella*, e.g., *L. pneumonophila*, *Pneumocystis*, e.g., *P. carinii*; Enterobacteriaceae such as *Salmonella*, *Shigella*, *Escherichia* (e.g., *E. coli*, *E. coli*O157:H7); *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Morganella*, *Providencia*, *Yersinia*, and the like, e.g., *Salmonella*, e.g., *S. typhi* *S. paratyphi* A, B (*S. schottmuelleri*), and C (*S. hirschfeldii*), *S. dublin* *S. choleraesuis*, *S. enteritidis*, *S. typhimurium*, *S. heidelberg*, *S. newport*, *S. infantis*, *S. agona*, *S. montevideo*, and *S. saint-paul*; *Shigella* e.g., subgroups: A, B, C, and D, such as *S. flexneri*, *S. sonnei*, *S. boydii*, *S. dysenteriae*; *Proteus* (*P. mirabilis*, *P. vulgaris*, and *P. myxofaciens*), *Morganella* (*M. morganii*); *Providencia* (*P. rettgeri*, *P. alcalifaciens*, and *P. stuartii*); *Yersinia*, e.g., *Y. pestis*, *Y. enterocolitica*; *Haemophilus*, e.g., *H. influenzae*, *H. parainfluenzae* *H. aphrophilus*, *H. ducreyi*; *Brucella*, e.g., *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*; *Francisella*, e.g., *F. tularensis*; *Pseudomonas*, e.g., *P. aeruginosa*, *P. paucimobilis*, *P. putida*, *P. fluorescens*, *P. acidovorans*, *Burkholderia* (*Pseudomonas*) *pseudomallei*, *Burkholderia mallei*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia*; *Campylobacter*, e.g., *C. fetus fetus*, *C. jejuni*, *C. pylori* (*Helicobacter pylori*); *Vibrio*, e.g., *V. cholerae*, *V. parahaemolyticus*, *V. mimicus*, *V. alginolyticus*, *V. hollisae*, *V. vulnificus*, and the nonagglutinable vibrios; *Clostridia*, e.g., *C. perfringens*, *C. tetani*, *C. difficile*, *C. botulinum*; *Actinomyces*, e.g., *A. israelii*; *Bacteroides*, e.g., *B. fragilis*, *B. thetaiotaomicron*, *B. distasonis*, *B. vulgatus*, *B. ovatus*, *B. caccae*, and *B. merdae*; *Prevotella*, e.g., *P. melaninogenica*; genus *Fusobacterium*; *Treponema*, e.g. *T. pallidum* subspecies *endemicum*, *T. pallidum* subspecies *pertenue*, *T. carateum*, and *T. pallidum* subspecies *pallidum*; genus *Borrelia*, e.g., *B. burgdorferi*; genus *Leptospira*; *Streptobacillus*, e.g., *S. moniliformis*; *Spirillum*, e.g., *S. minus*; *Mycobacterium*, e.g., *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. avium* *M. intracellulare*, *M. kansasii*, *M. xenopi*, *M. marinum*, *M. ulcerans*, the *M. fortuitum* complex (*M. fortuitum* and *M. chelonae*), *M. leprae*, *M. asiaticum*, *M. chelonae* subsp. *abscessus*, *M. fallax*, *M. fortuitum*, *M. malmoense*, *M. shimoidei*, *M. simiae*, *M. szulgai*, *M. xenopi*; *Mycoplasma*, e.g., *M. hominis*, *M. orale*, *M. salivarium*, *M. fermentans*, *M. pneumoniae*, *M. bovis*, *M. tuberculosis*, *M. avium*, *M. leprae*; *Mycoplasma*, e.g., *M. genitalium*; *Ureaplasma*, e.g., *U. urealyticum*; *Trichomonas*, e.g., *T. vaginalis*; *Cryptococcus*, e.g., *C. neoformans*; *Histoplasma*, e.g., *H. capsulatum*; *Candida*, e.g., *C. albicans*; *Aspergillus* sp; *Coccidioides*, e.g., *C. immitis*; *Blastomyces*, e.g. *B. dermatitidis*; *Paracoccidioides*, e.g., *P. brasiliensis*; *Penicillium*, e.g., *P. marneffeii*; *Sporothrix*, e.g., *S. schenckii*; *Rhizopus*, *Rhizomucor*, *Absidia*, and *Basidiobolus*; diseases caused by *Bipolaris*, *Cladophialophora*, *Cladosporium*, *Drechslera*, *Exophiala*, *Fonsecaea*, *Phialophora*, *Xylomyces*, *Ochroconis*, *Rhinoctadiella*, *Scolecobasidium*, and *Wangiella*; *Trichosporon*, e.g., *T. beigeli*; *Blastoschizomyces*, e.g., *B. capitatus*; *Plasmodium*, e.g., *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*; *Babesia* sp; protozoa of the genus *Trypanosoma*, e.g., *T. cruzi*; *Leishmania*, e.g., *L. donovani*, *L. major* *L. tropica*, *L. mexicana*, *L. braziliensis*, *L. viannia braziliensis*; *Toxoplasma*, e.g., *T. gondii*; Amoebas of the genera *Naegleria* or *Acanthamoeba*; *Entamoeba histolytica*; *Giardia lamblia*; genus *Cryptosporidium*, e.g., *C. parvum*; *Isospora belli*; *Cyclospora cayetanensis*; *Ascaris lumbricoides*; *Trichuris trichiura*; *Ancylostoma duodenale* or *Necator americanus*; *Strongyloides stercoralis* *Toxocara*, e.g., *T. canis*, *T.*

cati; *Baylisascaris*, e.g., *B. procyonis*; *Trichinella*, e.g., *T. spiralis*; *Dracunculus*, e.g., *D. medinensis*; genus *Filarioidea*; *Wuchereria bancrofti*; *Brugia*, e.g., *B. malayi*, or *B. timori*; *Onchocerca volvulus*; *Loa loa*; *Dirofilaria immitis*; genus *Schistosoma*, e.g., *S. japonicum*, *S. mansoni*, *S. mekongi*, *S. intercalatum*, *S. haematobium*; *Paragonimus*, e.g., *P. westermani*, *P. skriabini*; *Clonorchis sinensis*; *Fasciola hepatica*; *Opisthorchis* sp; *Fasciolopsis buski*; *Diphyllobothrium latum*; *Taenia*, e.g., *T. saginata*, *T. solium*; *Echinococcus*, e.g., *E. granulosus*, *E. multilocularis*; Picornaviruses, rhinoviruses echoviruses, coxsackieviruses, influenza virus; paramyxoviruses, e.g., types 1, 2, 3, and 4; adenoviruses; Herpesviruses, e.g., HSV-1 and HSV-2; varicella-zoster virus; human T-lymphotrophic virus (type I and type II); Arboviruses and Arnaviruses; Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae; Flavivirus; Hantavirus; Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis]); Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo]); Smallpox (variola); retroviruses e.g., human immunodeficiency viruses 1 and 2; human papillomavirus [HPV] types 6, 11, 16, 18, 31, 33, and 35.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organisms selected from the group consisting of *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter Baumannii*, *Serratia marcescens*, *Enterobacter aerogenes*, *Enterococcus faecium*, vancomycin-resistant *enterococcus* (VRE), *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*(MRSA), *Streptococcus viridans*, *Listeria monocytogenes*, *Enterococcus* spp., *Streptococcus* Group B, *Streptococcus* Group C, *Streptococcus* Group G, *Streptococcus* Group F, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Gardenerella vaginalis*, *Micrococcus* spp., *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Moraxella catarrhalis*, *Salmonella* spp., *Chlamydia trachomatis*, *Peptostreptococcus productus*, *Peptostreptococcus anaerobius*, *Lactobacillus fermentum*, *Eubacterium lentum*, *Candida glabrata*, *Candida albicans*, *Chlamydia* spp., *Campylobacter* spp., *Salmonella* spp., smallpox (variola major), *Yersinia pestis*, Herpes Simplex Virus I (HSV I), and Herpes Simplex Virus II (HSV II).

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of Group B *Streptococcus*.

Carrying out PCR on a PCR-ready sample can include heating the PCR reagent mixture and the neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample; contacting the neutralized polynucleotide sample or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; independently contacting each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide; and/or contacting the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence.

In various embodiments, a method of carrying out PCR on a sample can further include one or more of the following steps: heating the biological sample in the microfluidic

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cartridge; pressurizing the biological sample in the microfluidic cartridge at a pressure differential compared to ambient pressure of between about 20 kilopascals and 200 kilopascals, or in some embodiments between about 70 kilopascals and 110 kilopascals.

In various embodiments, a method of using the apparatus described herein can further include one or more of the following steps: determining the presence of a polynucleotide sequence in the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; determining a contaminated result if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof; and/or in some embodiments, wherein the PCR reagent mixture further comprises a positive control plasmid and a plasmid probe selective for at least a portion of the plasmid, the method further including determining a PCR reaction has occurred if the plasmid probe is detected. Fluorescence Detection System, Including Lenses and Filters, and Multiple Parallel Detection for a Multi-Lane Cartridge

A miniaturized, highly sensitive fluorescence detection system can be incorporated for monitoring fluorescence from the biochemical reactions that are the basis of nucleic acid amplification methods such as PCR.

Accordingly, another aspect of the apparatus includes a system for monitoring fluorescence from biochemical reactions. The system can be, for example, an optical detector having a light source (for example an LED) that selectively emits light in an absorption band of a fluorescent dye, lenses for focusing the light, and a light detector (for example a photodiode) that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. Alternatively, the optical detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye (a fluorogenic probe) and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes at a plurality of different locations of, for example, a microfluidic cartridge, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof.

In some embodiments, a given detector for use with the apparatus described herein is capable of detecting a fluorescence signal from nanoliter scale PCR reactions. Advantageously, the detector is formed from inexpensive components, having no moving parts. The detector is also configured to mate with a microfluidic cartridge as further described herein, and is also preferably part of a pressure application system, such as a sliding lid, that keeps the cartridge in place. The detector further has potential for 2 or 3 color detection and is controlled by software, preferably custom software, configured to sample information from the detector.

FIGS. 57-59 depict an embodiment of a highly sensitive fluorescence detection system including light emitting diodes (LED's), photodiodes, and filters/lenses for monitoring, in real-time, one or more fluorescent signals emanating from the microfluidic cartridge. The embodiment in FIGS. 57-59 has a two-color detection system having a modular

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design that mates with a single lane microfluidic cartridge. The detector comprises two LED's (blue and red, respectively) and two photodiodes. The two LED's are configured to transmit a beam of focused light on to a particular region of the cartridge. The two photodiodes are configured to receive light that is emitted from the region of the cartridge. One photodiode is configured to detect emitted red light, and the other photodiode is configured to detect emitted blue light.

FIGS. 60 and 61 show an exemplary read-head comprising a multiplexed 2 color detection system, such as multiple instances of a detection system shown in FIGS. 57-59, that is configured to mate with a multi-lane microfluidic cartridge. FIG. 60 shows a view of the exterior of a multiplexed read-head. FIG. 61 is an exploded view that shows how various detectors are configured within an exemplary multiplexed read head, and in communication with an electronic circuit board.

The module in FIGS. 60 and 61 is configured to detect fluorescence from each lane of a 12-lane cartridge, and therefore comprises 24 independently controllable detectors, arranged as 12 pairs of identical detection elements. Each pair of elements is then capable of dual-color detection of a pre-determined set of fluorescent probes. It would be understood by one of ordinary skill in the art that other numbers of pairs of detectors are consistent with the apparatus described herein. For example, 4, 6, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 pairs are also consistent and can be configured according to methods and criteria understood by one of ordinary skill in the art.

Exemplary Optics Assembly

In an exemplary embodiment, the optical chassis/pressure assembly is housed in an enclosure (made of plastic in certain embodiments) that can be positioned to cover a multi-lane microfluidic cartridge. The enclosure can optionally have a handle that can be easily grasped by a user, and is guided for smooth and easy pushing and pulling. The handle may also serve as a pressure-locking device. The enclosure's horizontal position is sensed in both the all-open and in the all-forward position, and reported to controlling software. The enclosure and optical chassis pressure assembly registers with a heater cassette module positioned underneath a microfluidic cartridge to within 0.010". A close fit is important for proper heater/cartridge interface connections. The enclosure assembly does not degrade in performance over a life of 10,000 cycles, where a cycle is defined as: beginning with the slider in the back position, and sliding forward then locking the handle down on a cartridge, unlocking the handle and returning it to the original back position. All optical path parts should be non-reflective (anodized, painted, molded, etc.) and do not lose this feature for 10,000 cycles. The optics unit is unaffected by a light intensity of $\leq 9,000$ foot-candles from a source placed 12" from the instrument at angles where light penetration is most likely to occur. No degradation of performance is measured at the photo-detector after 10,000 cycles.

When fabricating a detector assembly, a single channel is made that houses two LED sources (blue and amber) and two additional channels that house one photodiode detector each (Four total bored holes). The two paired channels (source and detector) are oriented 43° from each other, measured from the optical axis and are in-line with the other paired channels that are at the same 43° orientation. The holes bored in the optical chassis contain filters and lenses with appropriate spacers, the specifications of which are further described herein. The LED's are held in place to prevent movement as the mechanical alignment is important

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for good source illumination. The LED's are preferably twisted until the two "hot spots" are aligned with the reading channels on the cartridge. This position must be maintained until the LED's cannot be moved. The optical chassis can be made of aluminum and be black anodized. The bottom pressure surface of the optical chassis is flat to ± 0.001 " across the entire surface. The optical chassis is center-balanced such that the center of the optical chassis force is close to the center of the reagent cartridge. The pressure assembly (bottom of the optical chassis) provides uniform pressure of a minimum of 1 psi across all heater sections of the reagent cartridge. The optical assembly can be moved away from the reagent cartridge area for cartridge removal and placement. Appropriate grounding of the optical chassis is preferred to prevent spurious signals to emanate to the optic PCB.

The LED light sources (amber and blue) are incident on a microfluidic cartridge through a band pass filter and a focusing lens. These LED light sources have a minimum output of 2800 millicandles (blue) and 5600 millicandles (Green), and the center wavelengths are 470 (blue) and 575 (amber) nanometers, with a half band width of no more than 75 nanometers.

The LED light excites at least one fluorescent molecule (initially attached to an oligonucleotide probe) in a single chamber on a cartridge, causing it to fluoresce. This fluorescence will normally be efficiently blocked by a closely spaced quencher molecule. DNA amplification via TAQ enzyme will separate the fluorescent and quenching molecules from the oligonucleotide probe, disabling the quenching. DNA amplification will only occur if the probe's target molecule (a DNA sequence) is present in the sample chamber. Fluorescence occurs when a certain wavelength strikes the target molecule. The emitted light is not the same as the incident light. Blue incident light is blocked from the detector by the green only emission filter. Green incident light similarly is blocked from the detector by the yellow emission filter. The fluorescent light is captured and travels via a pathway into a focusing lens, through a filter and onto a very sensitive photodiode. The amount of light detected increases as the amount of the DNA amplification increases. The signal will vary with fluorescent dye used, but background noise should be less than 1 mV peak-to-peak. The photo-detector, which can be permanently mounted to the optical chassis in a fixed position, should be stable for 5 years or 10,000 cycles, and should be sensitive to extremely low light levels, and have a dark value of no more than 60 mV. Additionally, the photo-detector must be commercially available for at least 10 years. The lenses are Plano-convex (6 mm detector, and 12 mm source focal length) with the flat side toward the test cartridge on both lenses. The filters should remain stable over normal operating humidity and temperature ranges.

The filters, e.g., supplied by Omega Optical (Brattleboro, Vt. 05301), are a substrate of optical glass with a surface quality of F/F per Mil-C-48497A. The individual filters have a diameter of 6.0 ± 0.1 mm, a thickness of 6.0 ± 0.1 mm, and the AOI and $\frac{1}{2}$ cone AOI is 0 degrees and +8 degrees, respectively. The clear aperture is ≥ 4 mm diameter and the edge treatment is blackened prior to mounting in a black, anodized metal ring. The FITC exciter filters is supplied by, e.g., Omega Optical (PN 481 AF30-RED-EXC). They have a cut-off frequency of 466 ± 4 nm and a cut-on frequency of 496 ± 4 nm. Transmission is $\geq 65\%$ peak and blocking is: $\geq OD8$ in theory from 503 to 580 nm, $\geq OD5$ from 501-650 nm, $\geq OD4$ avg. over 651-1000 nm, and $\geq OD4$ UV-439 nm. The FITC emitter filters is supplied by, e.g.,

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Omega Optical (PN 534AF40-RED-EM). They will have a cut-off frequency of 514 ± 2 nm and a cut-on frequency of 554 ± 4 nm. Transmission is $\geq 70\%$ peak and blocking is: $\geq OD8$ in theory from 400 to 504 nm, $\geq OD5$ UV-507 nm, and $\geq OD4$ avg. 593-765 nm. The amber exciter filters are supplied by, e.g., Omega Optical (PN 582AF25-RED-EXC). They have a cut-off frequency of 594 ± 5 nm and a cut-on frequency of 569 ± 5 nm. Transmission is $\geq 70\%$ peak and blocking is: $\geq OD8$ in theory from 600 to 700 nm, $\geq OD5$ 600-900 nm, and $\geq OD4$ UV-548 nm. The amber emitter filters are supplied by, e.g., Omega Optical (PN 627AF30-RED-EM). They have a cut-off frequency of 642 ± 5 nm and a cut-on frequency of 612 ± 5 nm. Transmission is $\geq 70\%$ peak and blocking is: $\geq OD8$ in theory from 550 to 600 nm, $\geq OD5$ UV-605 nm, and $\geq OD5$ avg. 667-900 nm. The spacers should be inert and temperature stable throughout the entire operating range and should maintain the filters in strict position and alignment. The epoxy used should have optically black and opaque material and dry solid with no tacky residue. Additionally, it should have temperature and moisture stability, exert no pressure on the held components, and should mount the PCB in such a way that it is fixed and stable with no chances of rotation or vertical height changes. 50% of illumination shall fall on the sample plane within an area 0.1" (2.5 mm) wide by 0.3" (7.5 mm) along axis of the detection channel. Fluorescence of the control chip should not change more than 0.5% of the measured signal per 0.001" of height though a region ± 0.010 from the nominal height of the control chip.

An exemplary optics board is shown in FIG. 62, and is used to detect and amplify the fluorescent signature of a successful chemical reaction on a micro-fluidic cartridge, and controls the intensity of LED's using pulse-width modulation (PWM) to illuminate the cartridge sample over up to four channels, each with two color options. Additionally, it receives instructions and sends results data back over an LVDS (low-voltage differential signaling) SPI (serial peripheral interface). The power board systems include: a +12V input; and +3.3V, +3.6V, +5V, and -5V outputs, configured as follows: the +3.3V output contains a linear regulator, is used to power the LVDS interface, should maintain a $\pm 5\%$ accuracy, and supply an output current of 0.35 A; the +3.6V output contains a linear regulator, is used to power the MSP430, should maintain a $\pm 5\%$ accuracy, and supply an output current of 0.35 A; the +5V output contains a linear regulator, is used to power the plus rail for op-amps, should maintain a $\pm 5\%$ accuracy, and supply an output current of 0.35 A; the -5V output receives its power from the +5V supply, is used to power the minus rail for op-amps and for the photo-detector bias, should maintain a $\pm 1\%$ voltage accuracy, and supply an output current of 6.25 mA $\pm 10\%$. Additionally, the power board has an 80 ohm source resistance, and the main board software can enable/disable the regulator outputs.

The main board interface uses a single channel of the LVDS standard to communicate between boards. This takes place using SPI signaling over the LVDS interface which is connected to the main SPI port of the control processor. The interface also contains a serial port for in-system programming.

The exemplary optical detection system of FIG. 62 consists of a control processor, LED drivers, and a photo-detection system. In the exemplary embodiment, the control processor is a T1 MSP430F1611 consisting of a dual SPI (one for main board interface and one for ADC interface) and extended SRAM for data storage. It has the functions of power monitoring, PWM LED control, and SPI linking to

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the ADC and main board. The LED drivers contain NPN transistor switches, are connected to the PWM outputs of the control processor, can sink 10 mA@12V per LED (80 mA total), and are single channel with 2 LEDs (one of each color) connected to each. The photo-detection system has two channels and consists of a photo-detector, high-sensitivity photo-diode detector, high gain current to voltage converter, unity gain voltage inverting amplifier, and an ADC. Additionally it contains a 16 channel Sigma-delta (only utilizing the first 8 channels) which is connected to the second SPI port of the control processor. It would be understood by one of ordinary skill in the art that other choices and combinations of elements can be brought together to make a functioning detection system consistent with the description herein.

Additional Advantages and Features of the Technology Herein

The use of a disposable process chamber, having surface coating and material properties to allow low volume, and open tube heated release to maximize sample concentration in lowest volume possible.

The integrated magnetic heat separator that allows multiple samples to be heated independently but separated using a single moveable magnet platform.

A reader/tray design that allows easy placement of microfluidic cartridge and multiple sample pipetting of liquid using a robotic dispenser in one position; relative displacement to another location and pressure application for subsequent rapid heat incubation steps and optical detection. The bottom surface of the cartridge mates with the heating surface. Furthermore, it is typically easier to move a cartridge and heater in and out of position than a detector.

A moveable readhead design for fluorescence detection from microfluidic PCR channels.

Aspects of the holder, such as a unitized disposable strip, that include the presence of sealed lyophilized reagents as well as liquids sealed in close proximity, which is normally hard to achieve. The laminates deployed herein make storage easier.

The holder permits snapping of multiple ASR tubes, and associated liquid dispensing processes that minimizes cross-sample contamination but multiple PCR preparations to be performed from a single clinical sample.

Software features allow a user to either get results from all 24 samples as quickly as possible or the first 12 samples as quickly as possible and the next 12 later.

The preparatory and diagnostic instruments described herein enables different sample types (such as blood, urine, swab, etc.) to be all processed at the same time even though each may require different temperatures, times or chemical reagents. This is achieved in part by using individualized but compatible holders.

Automatic feeding of microfluidic cartridges into a PCR reader via a cartridge autoloader saves a user time and leads to increased efficiency of overall operation.

Piercing through foil over a liquid tube and reliable way of picking up liquid.

A moveable read-head that has the pumps, sensors (pipette detection, force sensing), sample identification verifier, etc., moving with it, and therefore minimizes the number of control lines that move across the instrument during use.

Accurate and rapid alignment of pipette tips with cartridge inlet holes using a motorized alignment plate.

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EXAMPLES

Example 1: Reagent Holder

An exemplary reagent holder consistent with the description herein has the following dimensions and capacities:

180 mm long×22 mm wide×100 mm tall;

Made from Polypropylene.

One snapped-in low binding 1.7 ml tube that functions as a process tube.

3 built-in tubes that function as receptacles for reagents, as follows:

One tube containing 200-1000 µl of wash buffer (0.1 mM Tris, pH 8).

One tube containing 200-1000 µl of release solution (40 mM NaOH).

One tube containing 200-1000 µl of neutralization solution (330 mM Tris, pH 8.0).

One built-in tube that functions as a waste chamber (will hold ~4 ml of liquid waste).

3 receptacles to accept containers for solid reagents. Snap-in 0.3 ml or 0.65 ml PCR tubes (which are typically stored separately from the reagent holder) are placed in each of these locations, and contain, respectively:

lyophilized sample preparation reagents (lysis enzyme mix and magnetic affinity beads).

First lyophilized PCR master mix, probes and primers for a first target analyte detection.

Second lyophilized PCR master mix, probes and primers for a second target analyte detection (only offered in select cases, such as detection of *Chlamydia* and *Gonorrhea* from urine).

4 pipette tips located in 4 respective sockets.

Pipette tip Sheath: The pipette tips have a sheath/drip tray underneath to help capture any drip from the pipette tips after being used, and also to prevent unwanted contamination of the instrument.

Handle and Flex-Lock allows easy insertion, removal, and positive location of strip in rack.

One or more labels: positioned upward facing to facilitate ease of reading by eye and/or, e.g., a bar-code reader, the one or more labels containing human and machine readable information pertaining to the analysis to be performed.

It is to be understood that these dimensions are exemplary. However, it is particularly desirable to ensure that a holder does not exceed these dimensions so that a rack and an apparatus that accommodates the reagent holder(s) does not become inconveniently large, and can be suitably situated in a laboratory, e.g., on a bench-top.

Example 2: Disposable Reagent Holder Manufacturing

Simple fixtures can be designed and machined to enable handling and processing of multiple strips. There are five steps that can be performed to produce this component. The disposable reagent holder will be placed in a fixture and filled with liquids using manual/electric-multiple pipetting. Immediately after dispensing all liquids into the strip, foil will be heat sealed to the plastic using exemplary heat seal equipment (Hix FH-3000-D Flat Head Press) and the foil trimmed as required. After heat sealing liquids on board, all pellets in tubes can be snapped into the strip, pipette tips can be inserted in their respective sockets, and a barcode label can be affixed. Desiccant packs can be placed into the blow molded or thermoformed rack designed to house 12 holders. Twelve disposable strips will be loaded into the rack and then sealed with foil. The sealed bag will be placed into a carton and labeled for shipping.

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Example 3: Foil-Sealing of Buffer Containing
Reagent Tubes

Tubes containing buffers have to be sealed with high moisture vapor barrier materials in order to retain the liquid over a long period of time. Disposable holders may need to have a shelf life of 1-2 years, and as such, they should not lose more than say 10-15% of the liquid volume over the time period, to maintain required volume of liquid, and to maintain the concentration of various molecules present in the solution. Moreover, the materials used for construction of the tube as well as the sealing laminate should not react with the liquid buffer. Special plastic laminates may provide the moisture barrier but they may have to be very thick (more than 300 μm thick), causing the piercing force to go up tremendously, or of special, expensive polymer (such as Aclar). Aluminum foils, even a thin foil of a few hundred angstrom provides an effective moisture barrier but bare aluminum reacts with some liquid buffers, such as sodium hydroxide, even an aluminum foil with a sprayed coating of a non-reactive polymer may not be able to withstand the corrosive vapors over a long time. They may react through tiny pin holes present in the coating and may fail as a barrier over time.

For these reasons, aluminum foils with a laminate structure have been identified as a suitable barrier, exemplary properties of which are described below:

1. Sealing

Heat seals to unitized polypropylene strip (sealing temp $\sim 170\text{-}180^\circ\text{C}$.)

No wrinkling, cracking and crazing of the foil after sealing

2. Moisture Vapor Transmission Rate (MVTR)

Loss of less than 10% liquid (20 microliters from a volume of 200 microliter) for a period of 1 year stored at ambient temperature and pressure. (effective area of transport is $\sim 63\text{ mm}^2$); Approximate MVTR $\sim 0.8\text{ cc/m}^2/\text{day}$

3. Chemistry

Ability to not react with 40 mM Sodium Hydroxide ($\text{pH} < 12.6$): foil should have a plastic laminate at least 15 microns thick closer to the sealed fluid.

Ability to not react with other buffers containing mild detergents

4. Puncture

Ability to puncture using a p1000 pipette with a force less than 3 lb

Before puncturing, a fully supported membrane 8 mm in diameter will not stretch more than 5 mm in the orthogonal direction

After puncturing, the foil should not seal the pipette tip around the circumference of the pipette.

5. Other Features

Pin-hole free

No bubbles in case of multi-laminate structures.

Example 4: Mechanism of Piercing Through a
Plasticized Laminate and Withdrawing Liquid
Buffer

The aluminum laminate containing a plastic film described elsewhere herein serves well for not reacting with corrosive reagents such as buffers containing NaOH, and having the favorable properties of pierceability and acting as a moisture barrier. However, it presents some additional difficulties during piercing. The aluminum foil tends to burst into an irregular polygonal pattern bigger than the diameter

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of the pipette, whereas the plastic film tends to wrap around the pipette tip with minimal gap between the pipette and the plastic film. The diameter of the hole in the plastic film is similar to the maximum diameter of the pipette that had crossed through the laminate. This wrapping of the pipette causes difficulty in dispensing and pipetting operations unless there is a vent hole allowing pressures to equilibrate between outside of the tube and the air inside of the tube.

A strategy for successful pipetting of fluid is as follows:

1. Pierce through the laminate structure and have the pipette go close to the bottom of the reagent tube so that the hole created in the laminate is almost as big as the maximum diameter of the pipette (e.g., $\sim 6\text{ mm}$ for a p1000 pipette)
2. Withdraw the pipette up a short distance so that a small annular vent hole is left between the pipette and the laminate. The p1000 pipette has a smallest outer diameter of 1 mm and maximum outer diameter of 6 mm and the conical section of the pipette is about 28 mm long. A vent hole thickness of a hundred microns is enough to create a reliable vent hole. This corresponds to the pipette inserted to a diameter of 5.8 mm, leaving an annulus of 0.1 mm around it.
3. Withdraw fluid from the tube. Note that the tube is designed to hold more fluid than is necessary to withdraw from it for a sample preparation procedure.

Example 5: Foil Piercing and Dissolution of
Lyophilized Reagents

The containers of lyophilized reagents provided in conjunction with a holder as described herein are typically sealed by a non-plasticized aluminum foil (i.e., not a laminate as is used to seal the reagent tubes). Aluminum foil bursts into an irregular polygonal pattern when pierced through a pipette and leaves an air vent even though the pipette is moved to the bottom of the tube. In order to save on reagents, it is desirable to dissolve the reagents and maximize the amount withdrawn from the tube. To accomplish this, a star-ridged (stellated) pattern is placed at the bottom of the container to maximize liquid volume withdrawn, and flow velocity in between the ridges.

Exemplary steps for dissolving and withdrawing fluid are as follows:

1. Pierce through the pipette and dispense the fluid away from the lyophilized material. If the pipette goes below the level of the lyophilized material, it will go into the pipette and may cause jamming of the liquid flow out of the pipette.
2. Let the lyophilized material dissolve for a few seconds.
3. Move pipette down touching the ridged-bottom of the tube
4. Perform an adequate number of suck and spit operations (4-10) to thoroughly mix the reagents with the liquid buffer.
5. Withdraw all the reagents and move pipette to dispense it into the next processing tube.

Example 6: Material and Surface Property of the
Lysis Tube

The material, surface properties, surface finish has a profound impact on the sensitivity of the assay performed. In clinical applications, DNA/RNA as low as 50 copies/sample ($\sim 1\text{ ml}$ volume) need to be positively detected in a background of billions of other molecules, some of which strongly inhibit PCR. In order to achieve these high level of

sensitivities, the surface of the reaction tube as well as the material of the surface has to be chosen to have minimal binding of polynucleotides. During the creation of the injection molding tool to create these plastic tubes, the inherent surfaces created by machining may have large surface area due to cutting marks as large as tens of microns of peaks and valleys. These surfaces have to be polished to SPI A1/A2 finish (mirror finish) to remove the microscopic surface irregularities. Moreover, the presence of these microscopic valleys will trap magnetic beads (0.5-2 μ) at unintended places and cause irregular performance. In addition to actual surface roughness, the surface hydrophobicity/surface molecules present may cause polynucleotides to stick at unintended places and reduce sensitivity of the overall test. In addition to the base material uses, such as homogenous polypropylene and other polymers, specific materials used during the molding of these tubes, such as mold release compounds or any additives to aid in the fabrication can have a profound impact on the performance of the reactions.

Example 7: Liquid Dispensing Head

Referring to FIGS. 18, 19A-C, and 63, an exemplary liquid dispenser is attached to a gantry, and receives instructions via electrical cable 1702. Barcode scanner 1701 is mounted on one face of the liquid dispenser. The gantry is mounted on a horizontal rail 1700 to provide movement in the x-direction. Not shown is an orthogonally disposed rail to provide movement in the y-direction. The liquid dispenser comprises a computer controlled motorized pump 1800 connected to fluid distribution manifold 1802 with related computer controlled valving 1801 and a 4-up pipetter with individually sprung heads 1803. The fluid distribution manifold has nine Lee Co. solenoid valves 1801 that control the flow of air through the pipette tips: two valves for each pipette, and an additional valve to vent the pump. Barcode reader 1701 enables positive detection of sample tubes, reagent disposables and microfluidic cartridges. The scanner is mounted to the z-axis so that it can be positioned to read the sample tube, strip, and cartridge barcodes.

Example 8: Integrated Heater/Separator

In FIG. 64 an exemplary integrated magnetic separator and heater assembly are shown. Magnetic separator 1400 and heater assembly 1401 were fabricated comprising twelve heat blocks aligned parallel to one another. Each heat block 1403 is made from aluminum, and has an L-shaped configuration having a U-shaped inlet for accepting a process chamber 1402. Each heat block 1403 is secured and connected by a metal strip 1408 and screws 1407. Magnet 1404 is a rectangular block Neodymium (or other permanent rare earth materials, K & J Magnetics, Forcefield Magnetics) disposed behind each heat block 1403 and mounted on a supporting member Gears 1406 communicate rotational energy from a motor (not shown) to cause the motorized shaft 1405 to raise and lower magnet 1404 relative to each heat block. The motor is computer-controlled to move the magnet at speeds of 1-20 mm/s. The device further comprises a printed circuit board (PCB) 1409 configured to cause the heater assembly to apply heat independently to each process chamber 1402 upon receipt of appropriate instructions. In the exemplary embodiment, the device also comprises a temperature sensor and a power resistor in conjunction with each heater block.

Example 9: Exemplary Software

Exemplary software accompanying use of the apparatus herein can include two broad parts—user interface and

device firmware. The user interface software can allow for aspects of interaction with the user such as—entering patient/sample information, monitoring test progress, error warnings, printing test results, uploading of results to databases and updating software. The device firmware can be the low level software that actually runs the test. The firmware can have a generic portion that can be test independent and a portion specific to the test being performed. The test specific portion (“protocol”) can specify the microfluidic operations and their order to accomplish the test.

FIGS. 65A and 65B shows screen captures from the programming interface and real time heat sensor and optical detector monitoring. This real time device performance monitoring is for testing purposes; not visible to the user in the final configuration.

User Interface:

A medical grade LCD and touch screen assembly can serve as the user interface via a graphical user interface providing easy operating and minor troubleshooting instructions. The LCD and touch screen have been specified to ensure compatibility of all surfaces with common cleaning agents. A barcode scanner integrated with the analyzer can be configured to scan the barcode off the cartridge (specifying cartridge type, lot #, expiry date) and if available the patient and user ID from one or more sample tubes.

Example 10: Exemplary Preparatory Apparatus

This product is an instrument that enables 24 clinical samples to be automatically processed to produce purified nucleic acid (DNA or RNA) in about half an hour (FIG. 66). Purified nucleic acid may be processed in a separate amplification-detection machine to detect the presence of certain target nucleic acids. Samples are processed in a unitized disposable strip, preloaded with sample preparation chemistries and final purified nucleic acids are dispensed into PCR tubes. Fluid handling is enabled by a pipetting head moved by a xyz gantry. (FIG. 67)

The System has the following sub-systems:

- Two sample processing racks, each rack processes up to 12 clinical samples in unitized disposable strips
- Magnetic separator-cum-tube heater assembly (24 heating stations)
- A four-probe liquid dispensing head
- 3-axis gantry to move the pipette head
- Peltier-cooled per-tube holding station to receive the purified DNA/RNA
- Control electronics
- Barcode reader

Operation: The user will get a work list for each sample, whether they want to extract DNA or RNA for each clinical sample. The sample tubes are placed on the rack and for each sample type (DNA or RNA), the user slides in a unitized reagent disposable (DNA or RNA processing) into corresponding lane of the rack. The unitized disposable (holder) will have all the sample prep reagents, process tubes as well as disposable pipettes already prepackaged in it. Once all disposables are loaded into the rack, the rack is placed in its location on the instrument. Open per tubes are placed in the peltier cooled tube holder where the final purified nucleic acid will be dispensed. The user then closes the door of the instrument and then starts the sample processing using the GUI (Graphical User Interface).

The instrument checks functionality of all subsystems and then reads the barcode of the sample tubes and the unitized reagent disposable. Any mismatch with a pre-existing work list is determined and errors are flagged, if necessary. The

instrument then goes through a series of liquid processing, heating, magnetic separations to complete the sample preparation steps for the each of the clinical sample and outputs the purified nucleic acid into the PCR tube. The basic steps involved in each sample processing are sample lysis, nucleic acid capture into magnetic affinity beads, washing of the magnetic beads to remove impurities, releasing the nucleic acid from the magnetic beads, neutralizing the released DNA and the dispensing into the final PCR tube. These tubes are maintained at 4° C. until all samples are processed and user takes away the tube for downstream processing of the nucleic acids.

Example 11: Exemplary Diagnostic Apparatus

The apparatus, in combination with the associated consumables, automatically performs all aspects of nucleic acid testing, including sample preparation, amplification, and detection for up to 48 samples per hour with the first 24 results available in less than an hour. The system is easy to use. An operator simply aliquots a portion of the patient sample into a dedicated tube that contains pre-packaged buffer. The operator places the dedicated tubes into positions on a sample rack. The operator then loads a disposable plastic reagent strip for the appropriate test in the rack. The only other consumable used in the apparatus are microfluidic PCR cartridges for conducting amplification and detection; each cartridge is capable of performing up to twelve PCR tests and two cartridges can be loaded into the analyzer at once. Should the apparatus require a new PCR cartridge, the analyzer will prompt the operator to load the cartridge. The analyzer will then prompt the operator to close the lid to initiate testing. All consumables and sample tubes are bar-coded for positive sample identification.

Sample lysis and DNA preparation, which will require approximately half an hour for a full run of 24 samples, is automatically performed by the analyzer's robotic and liquid handling components using protocols and reagents located in unitized, disposable plastic strips. The apparatus then automatically mixes the sample and PCR reagents, and injects the mixture into a cartridge that will be automatically processed by an integrated PCR machine. Rapid, real time PCR and detection requires less than 20 minutes. Results, which will be automatically available upon completion of PCR, are displayed on the instruments touch screen, printed or sent to the hospital information system, as specified by the user (or the user's supervisor).

Each instrument can process up to 24 samples at a time with a total throughput of 48 samples per hour after the first run. The analyzer is slightly less than 1 m wide and fits easily on a standard lab bench. All operations of the unit can be directed using the included barcode wand and touch screen. The analyzer can be interfaced with lab information systems, hospital networks, PCs, printers or keyboards through four USB interfaces and an Ethernet port.

The apparatus has the following characteristics.

Sensitivity: the apparatus will have a limit of detection of ~50 copies of DNA or RNA. (and may have a limit of detection as low as 25-30 copies of DNA/RNA).

Cost per Test: Due to the miniaturized, simplified nature of HandyLab reagents, cartridge and other consumables, the cost of goods per test will be relatively low and very competitive.

Automation: By contrast with current "automated" NAT systems, which all require some degree of reasonably extensive technologist interaction with the system, through the use of unitized tests and full integration of sample extrac-

tion, preparation, amplification and detection, the apparatus herein will offer a higher level of automation, and corresponding reduction in technologist time and required skill level, thereby favorably impacting overall labor costs.

Throughput: Throughput is defined as how many tests a system can conduct in a given amount of time. The apparatus will be capable of running 45 tests per hour, on average.

Time to First Result: In a hospital environment, time to first result is an especially important consideration. The apparatus will produce the first 24 results in less than an hour and an additional 24 results every half hour thereafter.

Random Access and STAT: Random access is the ability to run a variety of tests together in a single run and place samples in unassigned locations on the analyzer. Also, with chemistry and immunoassay systems, it is desirable to be able to add tests after a run has started. This is often referred to as "true random access" since the user is provided complete flexibility with regard to what tests can be run where on an analyzer and when a new sample can be added to a run. A STAT is a sample that requires as rapid a result as possible, and therefore is given priority in the testing cue on the analyzer. Today, essentially all chemistry and immunoassay analyzers are true random access and offer STAT capabilities. For NAT, however, very few systems offer any random access or STAT capabilities. The instrument herein will provide random access and STAT capabilities.

Menu: The number and type of tests available for the analyzer is a very important factor in choosing systems. The apparatus herein deploys a launch menu strategy that involves a mix of high volume, "standard" nucleic acid tests combined with novel, high value tests.

The apparatus enables 24 clinical samples to be automatically processed to purify nucleic acid, mix the purified DNA/RNA with PCR reagents and perform real-time PCR in microfluidic cartridge to provide sample to results in an hour. The exemplary apparatus has two PCR readers, each capable of running a 12 lane microfluidic cartridge using an optical system that has dedicated two-color optical detection system. FIG. 68, FIG. 69.

The apparatus has the following sub-systems:

- Two sample processing racks, each rack processes up to 12 clinical samples in unitized disposable strips
- Magnetic separator-cum-tube heater assembly (24 heating stations)

- A four-probe liquid dispensing head
- 3-axis gantry to move the pipette head

- Two PCR amplification-detection station, each capable of running a 12-lane microfluidic cartridge and dedicated 2-color optical detection system for each PCR lane.

- Control electronics

- Barcode reader

Pictures of exterior (face on) and interior are at FIGS. 70, 71, respectively.

Operation: The user will get a work list for each sample, whether they want to detect certain target analyte (such as GBS, *Chlamydia*, Gonorrhea, HSV) for each clinical sample. The sample tubes are placed on the rack and for each sample, the user slides in a unitized reagent disposable (analyte specific) into corresponding lane of the rack. The unitized disposable will have all the sample prep reagents, PCR reagents, process tubes as well as disposable pipettes already prepackaged in it. Once all disposables are loaded into the rack, the rack is placed in its location on the instrument. The user then places two 12-lane microfluidic PCR cartridges in the two trays of the PCR reader. The user then closes the door of the instrument and then starts the sample processing using the GUI (Graphical User Interface).

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The instrument checks functionality of all subsystems and then reads the barcode of the sample tubes, the unitized reagent disposables and the microfluidic cartridges. Any mismatch with a pre-existing work list is determined and errors are flagged, if necessary. The instrument then goes through a series of liquid processing, heating, magnetic separation to complete the sample preparation steps for the each of the clinical sample, mixes the purified nucleic acid with PCR reagents and dispenses the final mix into a lane of the microfluidic cartridges. After a microfluidic cartridge is loaded with the final PCR mix, the cartridge tray moves and aligns the cartridge in the reader and the optical detection system presses the cartridge against a microfluidic PCR heater surface. On-chip valves are actuated to close the reaction mix and then thermocycling is started to initiate the PCR reaction. At each cycle of PCR (up to 45 cycles), fluorescence from each PCR lane is detected by the optical detection system (2-colors per PCR lane) and final result is determined based on the threshold cycle (Ct).

The sample preparation steps for 24 samples are performed in about 40 minutes and the PCR reaction in about 20 minutes.

Sample Reader:

The Reader performs function testing of up to twelve properly prepared patient samples by PCR process (real-time PCR) when used in conjunction with HandyLab microfluidic (test) cartridges. Each unit will employ two Reader Modules for a total of up to twenty four tests. (FIGS. 72A and 72B) Operation of the Reader is designed for minimal customer interaction, requiring the loading and unloading of test cartridges only. During the "Load Disposables" sequence, the Reader will present a motor actuated tray for installation of the disposable cartridge. Sliding a small knob located in the front of the tray, a spring loaded protective cover will raise allowing the test cartridge to be nested properly in place. The cover is then lowered until the knob self-locks into the tray frame, securing the cartridge and preventing movement during the sample loading sequence.

Once the prepared samples have been dispensed via pipettes into the test cartridge, the tray will retract into the Reader, accurately positioning the test cartridge beneath the chassis of the optical assembly. The optical assembly will then be lowered by a captured screw driven stepper motor until contact is made with the test cartridge. At this point the test cartridge is located $\frac{1}{8}$ " above the target location on the heater assembly. As downward motion continues the test cartridge and its holder within the tray compress springs on the tray frame (these are used later to return the cartridge to its normal position and able to clear the encapsulated wire bonds located on the heater assembly during tray operation). Movement of the test cartridge and optical assembly is complete once contact with the heater assembly is made and a minimum of 2 psi is obtained across the two-thirds of the cartridge area about the PCR channels and their controlling gates. At this point the testing of the cartridge is performed using the heater assembly, measured with onboard optics, and controlled via software and electronics much in the same manner as currently operated on similar HandyLab instruments.

Once the functional testing is complete the main motor raises the optic assembly, releasing pressure on the test cartridge to return to its normal position. When commanded, the tray motor operating in a rack-and-pinion manner, presents the tray to the customer for cartridge removal and disposal. When the tray is in the extended position it is suspended above a support block located on the apparatus chassis. This block prevents the cartridge from

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sliding through the holder in the tray during loading and acts as a support while samples are pipetted into the disposable cartridge. Also provided in this support block is an assist lever to lift and grasp the disposable cartridge during removal. All components of the tray as well as support block and cartridge lift assist are removable by the customer, without tools, for cleaning and reinstalled easily.

Microfluidic PCR Heater Module:

The microfluidic PCR heater module comprises a glass wafer with photolithographically defined microheaters and sensors to accurately provide heat for actuation of valves and performing thermocycling required to perform a real-time PCR reaction. The wafer surface has dedicated individually controlled heating zones for each of the PCR lanes in the microfluidic cartridge. For a 12-up cartridge, there are 12 PCR zones and the 24-up cartridge, there are 24 PCR heating zones. The individual heaters and sensors are electrically connected to a Printed circuit board using gold or aluminum wire bonds. A thermally compliant encapsulant provides physical protection the wirebonds. While the present device is made on glass wafer, heaters can be fabricated on Si-on-Glass wafers and other polymeric substrates. Each substrate can have provide specific advantages related to its thermal and mechanical properties. Besides using photolithography process, such heating substrates can also be assembled using off-the-shelf electronic components such as power resistors, peltiers, transistors, maintaining the upper heating surface of each of the component to be at the same level to provide heating to a microfluidic cartridge. Temperature calibration values for each temperature sensor may be stored in a EEPROM or other memory devices co-located in the heater PCBoard.

12-Lane Cartridge:

This 12 channel cartridge is the same basic design that is described in U.S. provisional patent application Ser. No. 60/859,284, filed Nov. 14, 2006, with the following modifications: increase the PCR volume from 2 μ l to 4.5 μ l, leading to an increase in the input volume from 4 μ l to 6 μ l. The inlet holes are moved a few millimeters away from the edge of the cartridge to allow room for a 2 mm alignment ledge in the cartridge. A similar alignment ledge is also included on the other edge of the cartridge. (FIGS. 31A, 31B)

Enclosure:

The design of the apparatus enclosure must satisfy requirements: for customer safety during operation; provide access to power and communication interfaces; provide air entry, exit, and filtering; provide one-handed operation to open for installation and removal of materials; incorporate marketable aesthetics.

Cooling:

The cooling for the apparatus will be designed in conjunction with the enclosure and overall system to ensure all assemblies requiring air are within the flow path or receive diverted air.

The current concept is for the air inlet to be located on the bottom of the lower front panel. The air will then pass through a cleanable filter before entering the apparatus. Sheet metal components will direct the air to both the disposable racks and the main power supply. The air will then be directed through the card cages, around the readers and will exit through slots provided in the top of the enclosure.

Base Plate

The XYZ stage and frame are mounted to the base plate in a way where there will be no misalignment between the stage, cartridge and the disposable. The enclosure is

mounted to the base plate. Final design of the enclosure determines the bolt hole pattern for mounting. The backplane board mounts to the base plate with standoffs. All other boards mount to the backplane board. The disposable mounts on a rack which will be removable from the brackets mounted to the base plate. The reader brackets bolt to the base plate. Final design of the reader brackets determines the bolt hole pattern. The power supply mounts to the base plate. The base plate extends width and lengthwise under the entire instrument.

Example 12: Exemplary High-Efficiency Diagnostic Apparatus

A more highly multiplexed embodiment, also enables 24 clinical samples to be automatically processed to purify nucleic acids, mix the purified DNA/RNA with PCR reagents and perform real-time PCR in a microfluidic cartridge. This product has a single PCR reader, with a scanning read-head, capable of reading up to 4 different colors from each of the PCR lane. The cartridge has 24 PCR channels enabling a single cartridge to run all 24 clinical samples. In addition, this product has a cartridge autoloader, whereby the instrument automatically feeds the PCR reader from a pack of cartridges into the instrument and discard used cartridge into a waste tray. Diagrams are shown in FIGS. 73, and 74.

The apparatus has the following sub-systems:

Two sample processing racks, each rack processes up to 12 clinical samples in unitized disposable strips

Magnetic separator-cum-tube heater assembly (24 heating stations)

A four-probe liquid dispensing head

3-axis gantry to move the pipette head

A single PCR amplification-detection station capable of running a 24-lane microfluidic cartridge and a scanner unit to detect up to 4 colors from each PCR lane.

An autoloader unit to feed 24-lane microfluidic cartridge from a box into the PCR detection unit.

Control electronics

Barcode reader

Operation: The user will get a work list for each sample, whether they want to detect certain target analyte (such as GBS, *Chlamydia*, Gonorrhea, HSV) for each clinical sample. The sample tubes are placed on the rack and for each sample, the user slides in a unitized reagent disposable (analyte specific) into corresponding lane of the rack. The unitized disposable will have all the sample prep reagents, PCR reagents, process tubes as well as disposable pipettes already prepackaged in it. Once all disposables are loaded into the rack, the rack is placed in its location on the instrument. The user then closes the door of the instrument and then starts the sample processing using the GUI (Graphical User Interface).

The instrument checks functionality of all subsystems and then reads the barcode of the sample tubes, the unitized reagent disposables and presence of a 24-lane microfluidic cartridge. Any mismatch with a pre-existing work list is determined and errors are flagged, if necessary. The instrument then goes through a series of liquid processing, heating, magnetic separation to complete the sample preparation steps for the each of the clinical sample, mixes the purified nucleic acid with PCR reagents and dispenses the final mix into a lane of a 24-lane microfluidic cartridge. After the microfluidic cartridge is loaded with the final PCR mix, the cartridge is moved and aligned by an automated motorized pusher in the PCR reader. The optical detection system, then

presses the cartridge against a microfluidic PCR heater surface. On-chip valves are actuated to close the reaction mix and then thermo-cycling is started to initiate the PCR reaction. At each cycle of PCR (up to 45 cycles), fluorescence from each PCR lane is detected by the optical detection system (2-colors per PCR lane) and final result is determined based on the threshold cycle (Ct). The used cartridge is then pushed out automatically into a waste cartridge bin.

Microfluidic cartridges are stored in a cartridge pack (maximum 24 cartridges) and the instrument alerts the user to replace the cartridge pack and empty out the waste cartridge bin once all cartridges from the pack are used up. 24-Lane Cartridge

The 24-lane cartridge has two rows of 12 PCR lanes. Various views are shown in FIGS. 75-77. The cartridge has 3 layers, a laminate, a substrate, and a label. The label is shown in two pieces. Each Lane has a liquid inlet port, that interfaces with a disposable pipette; a 4 microliter PCR reaction chamber (1.5 mm wide, 300 microns deep and approximately 10 mm long), two microvalves on either side of the PCR reactor and outlet vent. Microvalves are normally open and close the channel on actuation. The outlet holes enables extra liquid (~1 μ l) to be contained in the fluidic channel incase more than 6 μ l of fluid is dispensed into the cartridge.

The inlet holes of the cartridge are made conical in shape and have a diameter of 3-6 mm at the top to ensure pipettes can be easily landed by the fluid dispensing head within the conical hole. Once the pipette lands within the cone, the conical shape guides the pipette and mechanically seals to provide error free dispensing or withdrawal of fluid into the cartridge. The bigger the holes, the better it is to align with the pipette, however, we need to maximize the number of inlet ports within the width of the cartridge as well as maintain the pitch between holes compatible with the inter-pipette distance. In this particular design, the inter-pipette distance is 18 mm and the distance between the loading holes in the cartridge is 8 mm. So lanes 1, 4, 7, 11 are pipetted into during one dispensing operation; lanes 2, 5, 8 and 12 in the next, and so on and so forth.

The height of the conical holes is kept lower than the height of the ledges in the cartridge to ensure the cartridges can be stacked on the ledges. The ledges on the two long edges of the cartridge enable stacking of the cartridges with minimal surface contact between two stacked cartridges and also help guide the cartridge into the reader from cartridge pack (cf. FIGS. 28-33).

Cartridge Autoloader

The Cartridge autoloader consists of a place for positively locking a pack of 24 microfluidic cartridges, pre-stacked in a spring-loaded box (e.g., FIG. 33). The box has structural elements on the sides to enable unidirectional positioning and locking of the box in the autoloader (FIG. 33). To load a new box, the user moves a sliding element to the left of the autoloader, places and pushes the box in the slot and releases the sliding lock to retain the box in its right location. Springs loaded at the bottom of the box helps push the box up when it needs to be replaced. The spiral spring present at the bottom of the cartridge pack pushed against the cartridges and is able to continually push the cartridge with a force of from 4 to 20 pounds.

The presence or absence of cartridges is detected by reading the barcode on top of the cartridge, if present.

To start a PCR run, the pipette head dispenses PCR reaction mix into the required number of lanes in the top cartridge in the autoloader (e.g., FIG. 28). The pusher pushes

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the top cartridge from the autoloader box into the two rails that guide the cartridge into the PCR reader. The cartridge is pushed to the calibrated location under the reader and then the optics block is moved down using a stepper motor to push the cartridge against the microheater surface. The bottom of the optics block (aperture plate) has projections on the sides to enable the cartridge to be accurately aligned against the apertures. The stepper motor pushes the cartridge to a pre-calibrated position (e.g., FIG. 30) which provides a minimum contact pressure of 1 psi on the heating surface of the micro fluidic cartridge.

After the PCR reaction is complete, the stepper motor moves up 5-10 mm away from the cartridge, relieves the contact pressure and enables the cartridge to travel in its guide rails. The pusher is activated and it pushes the cartridge out to the cartridge waste bin (e.g., FIG. 32). After this step, the pusher travels back to its home position. During its back travel, the pusher is able to rise above the top of the cartridge in the cartridge pack because it has a angular degree of freedom (see figure). A torsion spring ensures the pusher comes back to a horizontal position to enable it to push against the next cartridge in queue. The pusher is mechanically attached to a timing belt. The timing belt can be moved in either direction by turning a geared motor. The pusher is mounted to a slider arrangement to constrain it to move in only one axis (see, e.g., FIG. 31).

The cartridge pushing mechanism can also be made to not only push the cartridge from the autoloader box to the detection position, but also be used to move it back to the autoloading position. This will enable unused lanes in the microfluidic cartridge to be used in the next PCR run.

The cartridge autoloading box is also designed so that once all the cartridges are used, the box can be easily recycled or new cartridges added to it. This reduces the cost to the customer and the manufacturer.

Reader

The reader consists of an optical detection unit that can be pressed against a 24-lane microfluidic cartridge to optically interface with the PCR lanes as well as press the cartridge against a microfluidic heater substrate (FIG. 78). The bottom of the optics block has 24 apertures (two rows of 12 apertures) that is similar in dimension of the PCR reactors closest to the cartridge. The aperture plate is made of low fluorescent material, such as anodized black aluminum and during operation, minimized the total background fluorescence while maximizing the collection of fluorescent only from the PCR reactor (FIGS. 79A and 79B). The bottom of the aperture plate has two beveled edges that help align two edges of the cartridges appropriately such that the apertures line up with the PCR reactors. (FIGS. 80, 81)

The optical detection units (total of 8 detection units) are assembled and mounted onto a sliding rail inside the optical box so that the optical units can be scanned over the apertures (FIG. 82). Each unit is able to excite and focus a certain wavelength of light onto the PCR reactor and collect emitted fluorescence of particular wavelength into a photodetector. By using 4 different colors on the top 4 channels and repeating the 4 colors in the bottom channels, the entire scanner can scan up to 4 colors from each of the PCR lanes.

The optics block can be machined out of aluminum and anodized or injection molded using low fluorescence black plastic (FIG. 83). Injection molding can dramatically reduce the cost per unit and also make the assembly of optics easier. The designed units can be stacked back-to-back.

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Example 13: Exemplary Electronics for Use with Preparatory and Diagnostic Apparatuses as Described Herein

There are multiple independent software modules running on dedicated hardware: Described herein are exemplary specifications for the electronics used in the diagnostic (PCR) system. Additional information related to the PCR System is described elsewhere herein. In some embodiments, the PCR system includes eighteen printed circuit boards (PCBs) of nine different types. Referring to FIG. 86, the system can contain three multiplex (MUX) boards 100a-c, two of which (micro-heater MUX boards 100a-b), can each be used to run a micro-heater board 110a-b and the third (lysis heater MUX board 100c) can run one or more lysis heater boards 116 and 117. Each of the three MUX boards 100a-c can be controlled by a PC processor board via an Ethernet port. The two micro-heater boards 110a-b, each controlled by one of the MUX boards 100a-b, heat micro-zones on the microfluidic cartridge. In some embodiments, the system includes the two lysis heater boards 116 and 117, controlled by the lysis heater MUX board 100c, that heat lysis tubes in each of the two 12 sample racks.

Still referring to the PCBs included in the PCR system, the system can include two 12-channel optical detection boards 130a-b that can each detect optical fluorescence emitted by microfluidic cartridge chemistry. The optical detection boards can be controlled by one or more of the MUX boards 100a-c, using SPI, over a RS-422 interface. The system can include three motor control boards 140a-c, where one board (e.g., motor control board 140c) can control two magnetic separation motors (not shown), and the remaining two motor control boards (e.g., motor control boards 140a-b) can each run one reader tray motor (not shown) and one reader pressure motor (not shown). The motor control board running the magnetic separation motors (e.g., motor control board 140c) can be controlled via RS-485 interface from the lysis heater MUX board 100c and the two motor control boards 140a-b, each running one reader tray motor and one reader pressure motor, can be controlled via RS-485 interface by the micro-heater MUX boards 100a-b. The system can also include one PC processor board 150, which directs the overall sequencing of the system and can be controlled via external Ethernet and USB interfaces, and one PC processor base board 160, which provides internal interfaces for the PC processor board 150 to the remainder of the system and external interfaces. The system can include one main backplane 180 that interconnects all system boards, one motor control backplane 190 that interconnects the motor control boards 140a-c to the main backplane 180 and gantry (not shown), and two door sensor boards (not shown). One door sensor board provides an interconnect between the front door solenoid locks and the PC processor base board 160 and the other door sensor board provides an interconnect between the position sensors and the PC processor base board 160.

In some embodiments, the PCR system can include the off-the-shelf PC processor board 150. The PC processor board 150 can be an ETX form factor board that includes one 10/100 BASE-T Ethernet port, four USB ports, one analog VGA display port, two UART ports, one real-time clock, one parallel port, one PS2 keyboard port, one PS2 mouse port, stereo audio output, one IDE interface, and one I2C interface.

Referring to FIG. 87, the system can also include the PC processor base board 160 that includes a five port 10/100 BASE-T Ethernet bridge 161 for internal communication,

one of which can be connected to the 10/100 BASE-T Ethernet port of the PC Processor board **150**, another of which can be for diagnostic use (with a connector inside system cover), and three of which can communicate with the three MUX boards **100a-c** (one port for each MUX board **100a-c**) through the backplane **180**. The PC processor base board **160** can also include one USB to 10/100 BASE-T Ethernet port **162** for external Ethernet connections, one four port USB hub **163** for external connections, one external VGA connector **164**, one internal PS2 Mouse connector **165** (with a connector inside the system cover), and one internal PS2 Keyboard connector **166** (with a connector inside the system cover). The PC processor base board **160** can also include one internal stereo audio output **167** to on board speakers **168**, one internal CompactFlash connector **169** from an IDE port (with a connector inside the system cover), and one internal RS-232 interface **170** from a UART port (with a connector inside the system cover). Additional components included in the PC processor base board can include one internal RS-485 interface **171** from a UART port (with a connector inside the system cover), one internal temperature sensor **172** connected to the I2C interface, a battery for the real-time clock, and one parallel port **173**. The parallel port **173**, with connectors inside the system cover, can be internally connected as follows: one bit can be used to drive a high current low side switch for the two door solenoids, one bit can be used to generate a processor interrupt when either door sensor indicates that a door is opened, three bits can be used to program the EEPROM for configuring the Ethernet bridge **161**, and two bits can be connected to the Ethernet bridge management interface (not shown). The remaining bits can remain unassigned, with optional pull-up and pull-down resistors, and be brought out to a 10 pin Phoenix contact header.

Referring now to FIG. **88**, in some embodiments, the system can include the three MUX boards **100a-c**. While FIG. **88** depicts exemplary MUX board **100a**, each of the three MUX boards **100a-c** can include one or more of the features described below. The MUX board **100a** can include 96 pulse width modulated (PWM) controlled heating channels with heaters (about 33 ohm to about 150 ohm) heaters, that can support 20 or 24 volt (voltage externally provided) drives with a maximum current of about 800 mA. Each PWMs can be 12-bit with programmable start and stop points, can have 1 microsecond resolution, and can have a maximum duty cycle of about 75%. Each PWM period is programmable and is preferably set to 4 ms. The MUX boards can include a 4-wire RTD/heater connection with precision 1 mA sense current that can accommodate about 50 ohm to about 2500 ohm resistive temperature devices and have a measurement accuracy of ± 0.5 ohms. The thermal measurement sample period of the MUX boards is 32 ms including 8xPWM periods where 12 16-bit ADCs **101a** sample 8 successive channels each. The MUX address can be tagged to the ADC data.

Still referring to the MUX board **100a** depicted in FIG. **88**, an RS-422 optics board interface **102a** that interconnects over the backplane **180** and transfers data over a 4 wire SPI interface using local handshake signals and interrupts can be included on the MUX board **100a**. The MUX board **100a** can also include a 10/100 BASE-T Ethernet interface **103a** that interconnects to the system over the backplane **180** and an RS-485 interface **104a** that interconnects to the motor controller **140a** over the backplane **180**.

Referring now to FIG. **89**, in some embodiments, the system can include the optical detection boards **130a-b**. While FIG. **89** depicts exemplary optical detection board

130a, each of the optical detection boards **130a-b** can include one or more of the features described below. The optical detection board **130a** can include a 12-channel optics board design modified to use an RS-422 interface **131a**. The optical detection board **130a** can include 12-3 Watt, blue LEDs **132a** driven with about 6 V at about a 625 mA maximum. An exemplary LED used in the detection board **130a** is the Luxeon K2 emitter producing blue light at a wavelength of about 470 nm using about 27 mW@700 mA. The optical detection board **130a** can also include 12-3 Watt, amber LEDs **133a** driven with about 6 V at about a 625 mA maximum. An exemplary LED used in the detection board **130a** is the Luxcon K2 emitter producing amber light at a wavelength of about 590 nm using about 60 mW@700 mA. The detection board **130a** can include 24 lensed silicon photodiode detectors **134a**, an example of which is the Hamamatsu S2386-18L. These photodiode detectors **134a** are designed in a common TO-18 package. The detection board **130a** can also include an MSP430 processor **135a** with two PWM channels, one for the blue channel and one for the amber channel. The board **130a** can include individual LED enables **136a** and **137a** for each of the 12 color pairs set over the local SPI bus.

The PCR system can include a lysis heater board that provides and monitors heating to the lysis tubes. The heater board can include 12-70 Watt TO-247 power resistors (provide heat to the lysis tubes) designed to be fed 24V from one or more of the MUX boards **100a-c** (e.g., MUX board **100c**) and 12-2000 ohm Resistive Temperature Devices (RTD) to monitor the temperature of the lysis tubes. Optional resistors can be included to modify the full scale range of the RTDs. Included on the lysis heater board is a serial EEPROM that may hold a board serial number and can be used to identify the board type and revision level to software.

Referring now to FIG. **90**, in some embodiments, the system can include the micro-heater boards **110a-b**. While FIG. **90** depicts exemplary micro-heater board **110a**, each of the micro-heater boards **110a-b** can include one or more of the features described below. In some embodiments, the system can include the micro-heater board **110a** that includes a serial EEPROM and two optical interrupters. The serial EEPROM may hold a board serial number, can hold RTD calibration data, and can be used to identify the board type and revision level to software. The optical interrupters can be used to sense the reader tray position for the motor control board **140a** and sends the information to the Blue Cobra (motor controllers), which processes the information on the positions of the reader trays and accordingly controls the power to the emitters supplied by the motor control board **140a**. The micro-heater board **110a** can provide connections to the 96 channel micro-heater plate and control the 96 multiplexed heater/RTD devices to control cartridge feature temperature. The heater/RTD devices can be between about 50 ohms to about 500 ohms. The micro-heater board **110a** can bridge the RS-422 interface from, for example, the MUX board **100a** to the optical detection board **130a**. The connection from the micro-heater board **110a** to the MUX board **100a** is over the backplane **180**, while the connection to the optics board **130a** is over a 40 pin FFC cable.

Referring now to FIG. **91**, in some embodiments, the system can include the motor control boards **140a-c**. While FIG. **91** depicts exemplary motor control board **140a**, each of the motor control boards **140a-c** can include one or more of the features described below. In some embodiments, the system can include the motor control board **140a** that can control two micro-stepping motors **141a** and can be con-

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nected to the backplane **180** via a RS-485 interface. The output to the motors can be up to 24 V supplied externally through the backplane **180**. The output current can be jumper selectable. Exemplary output currents that can be selected via jumper settings can include about 700 mA, about 1.0 A, or 2.3 A. The motor control board **140a** includes open collector TTL interrupt output to the MUX board **100a** and flag inputs. The flag inputs can provide 1.5 V power output to the sensors and can be switched on and off by software.

Limit switches are placed on the extreme locations of each axis, e.g., x-minimum and x-maximum, that turns off the power to the motor driving that axis in case of a malfunction happens and the pipette head moves out of the designed working distance. Optional pull-up and pull-down are used with the output of the optical interrupters.)

In some embodiments, the system can include one or more interconnection boards, such as the main backplane **180**. The main backplane **180** can interconnect other PCBs, such as the MUX boards **100a-c**, PC processor base board **160**, and heater Interconnect boards. The main backplane **180** can cable to the motor control backplane **190** and to two lysis heater boards. The main backplane **180** can distribute power and signaling, implement 10/100 BASE-T Ethernet and RS-485 over the backplane **180**, and supplies voltages from an external connector. Exemplary voltages supplied include +3.3 V, +5.0 V, +12.0 V, -12.0 V, +20.0 V, and +24.0 V.

The system can include the motor control backplane **190** that can distribute power and signaling for all of the motor control boards **140a-c**. The motor control backplane **190** can supply +5.0 V and 24.0 V from an external connector. The motor control backplane **190** can include 1 slot for the RS-485 signaling from each of the two MUX boards **100a-b** (total of 2 slots), 6 slots for the RS-485 signaling from the lysis heater controlling MUX board **100c**, and one connector that provides RS-485 signaling and power to the gantry. The motor control backplane **190** can provide pull-up and pull-down resistors to handle floating buses.

In some embodiments, the system can include a heater interconnect board and a door sensor board. The heater interconnect board can connect the micro-heater boards **110a-b** to the main backplane **180** using a physical interconnect only (e.g., no active circuits). The door sensor board can provide a cable interface and mixing logic from the optical interrupters, which sense the door is open, and provide a mounting and cabling interface to the door lock solenoid,

Example 14: Exemplary Software for Use with Preparatory and Diagnostic Apparatuses as Described Herein

There are multiple independent software modules running on dedicated hardware:

- Reader (2);
- Sample-Prep (1);
- User Interface (1);
- Detector (2);
- Motor control (8)

Inter-module communication among is via an internal Ethernet bus, communication with the user interface is via a high speed SPI bus and communication with motor control via a RS485 serial bus.

The Reader and Sample-Prep software run on identical hardware and are as such identical incorporating the following functions:

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Script Engine (a parameterized form of a protocol) Protocol Engine

Temperature Control (Microfluidics, lysis, release)

Motor control (via external motor control modules).

Salient features of the motor control software are:

Command/reply in ASCII and addressing capability to allow daisy chaining of communication link.

Detection (via external detector modules) Detector module controls the LED illumination and photo detector digitization.

The user interface is implemented as a program running under Linux operating system on an embedded x86 compatible PC. The following functions are addressed:

Graphical User Interface

Test control and monitor

Test result storage and retrieval Network connectivity via Ethernet (to lab information systems)

USB interface

Printer

Scanner (Internal and external)

Keyboard

Mouse

Door lock and sense

Example 15: Exemplary Chemistry and Processes of Use

Chemistry Overview

The chemistry process centers around the detection and identification of organisms in a clinical specimen, by virtue of detecting nucleic acids from the organism in question. This involves isolation of nucleic acids from target organisms that are contained in a clinical specimen, followed by a process that will detect the presence of specific nucleic acid sequences. In addition to target detection, an internal positive control nucleic acid will be added to the collection buffer, and will be taken through the entire extraction and detection process along with target nucleic acids. This control will monitor the effectiveness of the entire process and will minimize the risk of having false negative results. Nucleic Acid Extraction and Purification:

Nucleic acid extraction procedures begin with the addition of a clinical specimen to a prepared specimen collection solution. This can be done either at a specimen collection site, or at the testing site. Two collection solution formats will be available: one for body fluids, and one for swab specimens. Collection solutions used at collection sites will serve as specimen transport solutions, and therefore, this solution must maintain specimen and analyte integrity.

The extraction and purification procedure, which is entirely automated, proceeds as follows:

Target organisms are lysed by heating the detergent-containing collection solution.

Magnetic beads, added to the specimen/collection solution mix, non-specifically bind all DNA that is released into the solution.

Magnetic beads are isolated and are washed to eliminate contaminants

DNA is released from the beads using high pH and heat.

DNA containing solution is removed and neutralized with a buffer

Nucleic Acid Amplification:

Nucleic acids that have been captured by magnetic beads, washed, released in high pH, and neutralized with buffer, are added to a mixture of buffers, salts, and enzymes that have been lyophilized in a tube. The mixture is rapidly rehydrated, and then a portion of the solution is loaded onto a

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microfluidic cartridge. The cartridge is then loaded into the amplification instrument module, which consists of a heating unit capable of thermal cycling, and an optical detection system. Detection of target nucleic acids proceeds as follows:

The liquid is sealed in a reaction chamber.

Rapid thermal cycling is used to potentiate the Polymerase Chain Reaction (PCR), which is used to amplify specific target DNA.

Amplified DNA fluoresces, and can be detected by optical sensors.

A fluorescent probe "tail" is incorporated into each amplified piece of DNA

At a specific temperature, the probe adopts a conformation that produces fluorescence (this is termed a "scorpion" reaction, see FIG. 84).

Fluorescence is detected and monitored throughout the reaction.

Extraction and Amplification/Detection Process:

Extensive bench-scale testing has been performed to optimize the nucleic acid extraction chemistry, including the collection buffer, the wash buffer formulation, the release solution formulation, and the PCR reagent mixes. The fully automated method of extraction, followed by 12-up PCR, was able to provide very high sensitivity consistently at 150 copies/sample.

Examples: *Chlamydia* in Urine (50/50); Gonorrhea in Urine; GBS in Plasma.

Various detection chemistries such as Taqman, Scorpion, SYBRg Green work reliably in the microfluidic cartridge. Reagent Manufacturing

Feasibility studies were conducted in order to determine whether PCR reagents could be lyophilized in PCR tubes besides the use of 2 µl lyophilized pellets. The studies have indicated that sensitivity of reactions performed using tube-lyophilized reagents is equivalent to that of wet reagents or 2 µl pellet reagents, so feasibility has been proven. Stability studies for this format indicate similar stability data. We have seen 2 microliter lyophilized PCR pellets to be stable to up to 2 years at room temperature, once sealed in nitrogen atmosphere.

Manufacturing Overview: Manufacturing the components of the system can be accomplished at HandyLab, Inc., Ann Arbor, Mich. The manufacturing task has been split into five areas that consist of: chemistry manufacture, disposable strip, collection kit, cartridge and analyzer.

Chemistry Manufacturing: There are currently seven individual, blended chemistry components identified for potential use with the system described herein. Mixing, blending and processing reagents/chemicals can be performed at HandyLab, Inc., with existing equipment already in place. Additional tooling and fixtures will be necessary as the product matures and we ramp to high volume production, but initial costs will be minimal.

Collection buffer, wash, release & neutralization liquids are simple recipes with very low risk, and can be made in large batches to keep labor costs of mixing/blending at or below targeted projections. They will be mixed and placed into intermediate containers for stock, and then issued to Disposable Strip Manufacturing for dispensing. Mature SOP's are in place from prior project activity.

Affinity Beads (AB) have good potential to be stored and used as a liquid in the strip, but design contingencies for using a lyophilized pellet are in place as a back up. It is critical to keep the beads suspended in solution during dispense. Dispense equipment (e.g., manufactured by Innovadyne) that provides agitation for continuous suspension

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during dispense has been identified for purchase once stability has been proven for liquid AB storage in the strip. The process to manufacture and magnetize the Affinity Beads spans a 9 hour cycle time to produce a batch of 2,000 aliquots, but that same time period can be used for scaled up recipe batches once we ramp into high volume production. This item has the highest labor content of all chemistry manufacture that is currently required for the apparatus.

PCR reagents/enzymes will be freeze-dried in our existing lyophilizing chamber (Virtis Genesis) but will not require spherical pellet formation. Instead, the mixture is being dispensed into, and then lyophilized, inside the end-use tube. First the chemistries are mixed per established SOPs, and then the following steps are performed to accomplish lyophilization: Individual tubes are placed into a rack/fixture, and the solution is dispensed into each, using existing equipment (EFD Ultra Dispense Station.). The filled rack will be placed inside a stainless steel airtight box (modified to accept stoppers in the lid,) and then placed into the lyophilization chamber and the drying cycle commences unattended. During lyophilization, the stoppers are in a raised position allowing air/nitrogen to circulate into, and moisture to exit the stainless box holding racks of vials. At the end of the cycle, the shelves of our lyophilization chamber lower to seat the stoppers into the lid, forming a seal while still inside the closed chamber, in a moisture free nitrogen atmosphere. The steel boxes are then removed from the chamber, and each rack inside shall be processed in a single operation to seal all vials in that rack. Immediately after sealing, the vials will be die cut from the foil in one operation, allowing individual vials to be forwarded to the Disposable Manufacturing area for placement into a strip. Internal Control will either be added to an existing solution, or will be dispensed into its own cavity in the manner of the collection buffer, wash, neutralization, and release solutions. If lyophilization is required, it will be accomplished in the same manner as the PCR chemistry, and later snapped into the strip. Shelf life stability studies are underway.

Collection Kit Manufacturing

The collection kit will be processed manually in house for initial quantities. Initial quantities will not require capital expenditures as we have all equipment necessary to enable us to meet projections through 2008. We will be using our existing equipment (EFD 754-SS Aseptic Valve & Valve-mate 7000 Digital Controller,) to fill the collection vial. The vials have a twist-on top that will be torqued, and the vial will have a proprietary 1D barcode on each vial. 24 vials will be placed into a reclosable plastic bag and placed into a carton for shipping.

Place vials into rack.

Dispense solution into vials.

Install and torque caps.

Label vials.

Bag vials and label bag.

Place vial bag and instructions/insert into carton, close and label.

Cartridge Manufacturing:

Existing semi-automatic equipment for laminating & waxing (Think & Tinker DF-4200, & Asymtek Axiom Heated Jet Platform, respectively,) will be utilized to meet all cartridge manufacture requirements. The footprint of the 12-up disposable is the same as the RTa10 cartridge, so additional fixtures are not necessary.

Laminate micro substrate & trim excess.

Fill valves with hot wax & inspect.

Apply label & barcode.

Band 24 pieces together,

Bag & seal banded cartridges, label bag.

Place bag & insert(s) into carton, seal and label.

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This portion of the product is relatively simple, although there is a difference between the automated (as used herein) and the stand-alone 12-up cartridge. Venting will not be required on the cartridge, which eliminates the most time consuming process for cartridge manufacture, along with the highest risk and highest cost for fully integrated automation. Over 1,000 pieces of the 12-up with venting have been successfully produced.

Example 16: Exemplary Chemistry Processes

Sample Pre-Processing

For Urine Sample: Take 0.5 ml of urine and mix it with 0.5 ml of HandyLab collection buffer. Filter the sample through HandyLab Inc.'s pre-filter (contains two membranes of 10 micron and 3 micron pore size). Place the sample tube in the position specified for the external sample tube in the 12-up rack.

For Plasma Sample: Take 0.5 ml of plasma and mix it with 0.5 ml of HandyLab collection buffer. Place the sample tube in the position specified for the external sample tube in the 12-up rack.

For GBS swab samples: Take the swab sample and dip it in 1 ml of HandyLab collection buffer. Place the sample tube in the position specified for the external sample tube in the 12-up rack.

The HandyLab sample collection buffer contains 50 mM Tris pH 7, 1% Triton X-100, 20 mM Citrate, 20 mM Borate, 100 mM EDTA, plus 1000 copies of positive control DNA.

Loading the Instrument and Starting Sample Processing

1. Load PCR tube containing PCR master mix in one of the specified snap-in location of the unitized disposable.
2. Load PCR tube containing PCR probes and primers for the target analyte under consideration in the specified location of the unitized disposable.
3. In case of two analyte test, load PCR tube containing probes and primers for second analyte in the specified location of the unitized disposable.
4. Load the unitized disposable in the 12-up rack in the same lane as the sample tube under consideration.
5. Prepare and load unitized reagent strips for other samples in consideration.
6. Load the 12-up rack in one of the locations in the instrument.
7. Load 12-up cartridge in the cartridge tray loading position.
8. Start operation.

Liquid Processing Steps

1. Using Pipette tip #1, the robot transfers the clinical sample from the external sample tube to the lysis tube of the unitized disposable strip.
2. Using the same pipette tip, the robot takes about 100 μ l of sample, mixes the lyophilized enzyme and affinity beads, transfers the reagents to the lysis tube. Mixing is performed in the lysis tube by 5 suck and dispense operations.
3. The robot places pipette tip #1 at its designated location in the unitized disposable strip.
4. Heat the lysis tube to 60 C and maintain it for 10 minutes.
5. After 5 minute of lysis, the robot picks up pipette tip #1 and mixes the contents by 3 suck and dispense operations.

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6. The robot places pipette tip #1 at its designated location in the unitized disposable strip.
7. After 10 minutes of lysis, a magnet is moved up the side of the lysis tube to a middle height of the sample and held at that position for a minute to capture all the magnetic beads against the wall the tube.
8. The magnet is brought down slowly to slide the captured beads close to the bottom (but not the bottom) of the tube.
9. Using pipette tip #2, aspirate all the liquid and dump it into the waste tube.
10. Aspirate a second time to remove as much liquid as possible from the lysis tube.
11. Using the same pipette tip #2, withdraw 100 μ l of wash buffer and dispense it in the lysis tube. During this dispense, the magnet is moved downwards, away from the lysis tube.
12. Perform 15 mix steps to thoroughly mix the magnetic beads with the wash buffer.
13. Wait for 30 seconds.
14. Move magnet up to capture the beads to the side and hold for 15 seconds.
15. Using pipette tip #2, aspirate wash buffer twice to remove as much liquid as possible and dump it back in the wash tube.
16. Move magnet down away from the lysis tube.
17. Place pipette tip #2 in its specified location of the unitized disposable strip.
18. Pick up a new pipette tip (tip #3) and withdraw 8-10 μ l of release buffer and dispense it over the beads in the lysis tube.
19. Wait for 1 minute and then perform 45 mixes.
20. Heat the release solution to 85° C. and maintain temperature for 5 minutes.
21. Place pipette tip #3 in its specified location of the unitized disposable strip.
22. Bring magnet up the tube, capture all the beads against the tube wall and move it up and away from the bottom of the tube.
23. Pick up a new pipette tip (tip #4) and withdraw all the release buffer from the lysis tube and then withdraw 3-10 μ l of neutralization buffer, mix it in the pipette tip and dispense it in the PCR tube. (In case of two analyte detections, dispense half of the neutralized DNA solution into first PCR tube and the rest of the solution in the second PCR tube.
24. Using pipette tip #4, mix the neutralized DNA with the lyophilized reagents by 4-5 suck and dispense operations and withdraw the entire solution in the pipette tip.
25. Using pipette tip #4, load 6 μ l of the final PCR solution in a lane of the 12-up cartridge.

The usage of pipette heads during various processes is shown schematically in FIGS. 85A-C.

Real-Time PCR

After all the appropriate PCR lanes of the PCR cartridge is loaded with final PCR solution, the tray containing the cartridge moves it in the PCR Analyzer. The Cartridge is pressed by the Optical detection read-head against the PCR heater. Heaters activate valves to close either ends of the PCR reactor and real-time thermocycling process starts. After completing appropriate PCR cycles (~45 cycles), the analyzer make a call whether the sample has the target DNA based on the output fluorescence data.

Pipette Detection

The pipette head has 4 infrared sensors for detecting the presence of pipettes. This is essential to ensure the computer positively knows that a pipette is present or missing. Since

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pipettes are picked up using mechanical forcing against the pipette and also dispensed using mechanical motion of a stripper plate, pipette sensing helps preventing errors that otherwise may happen.

Force Sensing of the Pipette Head

The multi-pipette head is assembled in such a way and a force sensor interfaced with it so that any time the pipette head seats against the disposable pipette(s) or the picked pipettes are forced through the laminate in the reagent disposable or the pipette is forced against the bottom of the tubes in the reagent disposable, an upward force acts on the pipette head through the pipette holding nozzle or the pipettes itself. The entire head is pivoted, as shown in Figure and any force acting on the head causes a set-screw on the upper part of the head to press against a force sensor. This force sensor is calibrated for vertical displacement of the head against a non-moving surface. Using this calibration, it can be determined when to stop moving the head in the z-direction to detect whether pipettes are properly seated or if pipettes hit tube bottoms.

Alignment of Pipette Tips while Loading PCR Reagents into the Microfluidic Cartridge

The pipettes used in the apparatus can have volumes as small as 10 μ l to as large as 1 ml. Larger volume pipettes can be as long as 95 mm (p1000 pipette). When 4 long pipette tips are sprung from the head, even a 1° misalignment during seating can cause the tip to be off-center by 1.7 mm. As it is impossible to have perfect alignment of the tip both at the top where it is interfaced with the tip holder and the bottom, it becomes necessary to mechanically constrain all the tips at another location closer to the bottom. We have used the stripper plate, having a defined hole structure to use it to align all the tips. The stripper plate hole clears all the 4 pipette tips when they are picked up. After the tips are properly seated, the stripper plate is moved in the x-axis using a motor to move all the pipettes against the notch provided in the stripper plate (see FIG. 46b). Now all the pipettes land on the cartridge inlet holes with ease.

Sample Preparation Extensions

The current technology describes details of processing clinical samples to extract polynucleotides (DNA/RNA). The same product platform can be extended to process samples to extract proteins and other macromolecules by changing the affinity molecules present in the magnetic beads. The amplification-detection platform can also be used to perform other enzymatic reactions, such as immunoPCR, Reverse-transcriptase PCR, TMA, SDA, NASBA, LAMP, LCR, sequencing reactions etc. The sample preparation can also be used to prepare samples for highly multiplexed microarray detections as well.

Example 16: Exemplary Material for RNA-Affinity Matrix

An exemplary polynucleotide capture material preferentially retains polynucleotides such as RNA on its surface when placed in contact with a liquid medium that contains polynucleotides mixed with other species such as proteins and peptides that might inhibit subsequent detection or amplification of the polynucleotides.

The exemplary polynucleotide capture material is: Polyamidoamine (PAMAM) Generation 0, available from the Sigma-Aldrich Chemical Company ("Sigma-Aldrich"), product number 412368. PAMAM is a dendrimer whose molecules contain a mixture of primary and tertiary amine groups. PAMAM (Generation 0) has the structure shown herein.

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The PAMAM, during use, is immobilized on a solid support such as carboxylated beads, or magnetic beads. The polynucleotide capture material comprises polycationic molecules during an operation of polynucleotide capture. Affinity between the material and polynucleotides is high because polynucleotides such as DNA and RNA typically comprise polyanions in solution.

After polynucleotide molecules are captured on a surface of the material, and remaining inhibitors and other compounds in solution have been flushed away with an alkaline buffer solution, such as aqueous 0.1 mM Tris (pH 8.0), the polynucleotides may themselves be released from the surface of the material by, for example, washing the material with a second, more alkaline, buffer, such as Tris having a pH of 9.0.

Exemplary protocols for using PAMAM in nucleic acid testing are found in U.S. patent application Ser. No. 12/172,214 filed Jul. 11, 2008, incorporated herein by reference.

Example 17: Exemplary Material for DNA-Affinity Matrix

The exemplary polynucleotide capture material is: Polyethyleneimine (PEI), available from the Sigma-Aldrich Chemical Company ("Sigma-Aldrich"), product number 408719.

Exemplary protocols for using PEI in nucleic acid testing are found in U.S. patent application Ser. No. 12/172,208 filed Jul. 11, 2008, incorporated herein by reference.

Example 18: Exemplary Apparatus

Described herein are exemplary specifications for the mechanical design of the PCR system. In some embodiments, the system can be about 28.5 inches deep, or less, and about 43 inches wide, or less, and weight about 250 pounds or less. The system can be designed with a useful life of about 5 years (e.g., assuming 16,000 tests per year) and can be designed such that the sound level for this instrument (during operation) does not exceed 50 dB as measured 12 inches from the instrument in all ordinate directions. In some embodiments, the exterior of the system can be white with texture.

Referring to the overall system, in some embodiments, critical components of the system can remain orthogonal or parallel (as appropriate) to within 0.04 degrees. Exemplary critical components can include motion rails, pipettes, nozzles (e.g., axially as individual nozzles, linearly as an array of four nozzle centroids, or the like), lysis heaters, major edges of the installed cartridge holder in the reader drawer, the front face of the separation magnets, and the like. In the following descriptions, the X-axis (or X direction) refers to the axis extending from left to right when facing the front of the system, the Y-axis (or Y direction) refers to the axis extending from back to front when facing the front of the system, and the Z-axis (or Z direction) refers to the axis extending up from the bottom when facing the front of the system. As viewed from the top of the instrument, the centroid of the leftmost pipette nozzle on the Z-payload (as viewed from the front of the instrument) can be capable of unobstructed travel in the X direction from a point 80 mm from the outermost left baseplate edge to a point 608 mm from the outermost left baseplate edge and can be capable of unobstructed travel in the Y direction from a point 60 mm from the outermost front baseplate edge to a point 410 mm from the outermost front baseplate edge.

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Still referring to the system, as viewed from the front of the instrument, the bottom-most face of the pipette nozzles on the Z-payload can be capable of unobstructed travel in the Y direction from a point 156 mm above the top surface of the baseplate to a point 256 mm above the top surface of the baseplate. The 1 ml pipette tips can be capable of penetrating the foil covers included on disposable reagent strips. This penetration may not create contamination, affect the associated chemistries, or damage the pipette tips. Motions can be executed in such a manner as to eliminate mechanical hysteresis, as needed. Gantry motions can be optimized to prevent cross lane contamination and carryover. The rack can align the reagent strips to a tolerance of ± 0.010 inches in the X and Y directions.

Referring now to the gantry, in some embodiments, the gantry can consist of a stepper-motor actuated, belt/screw-driven cartesian robotic system. The gantry can be free to move, with or without attachments, above the modules that are forward of the rear facade and below the bottom-most horizontal face on the Z head, so long as the Z-payload is fully retracted. The gantry can be capable of travel speeds up to about 500 mm/sec in the X and Y directions and up to about 100 mm/sec in the Z direction. The accuracy and precision of the axis motions (e.g., with respect to the X, Y, and Z home sensors) can be 25 mm or better for each axis, and can be retained throughout the maintenance period. The axis drive belts may not leave residue in areas where PCR and samples are processed. The gantry can contain provisions for routing its own and all Z-payload wire harnesses back to the instrument. Belt tension on the X and Y axes can be set at 41.5 \pm 3.5 pounds.

Referring now to the Z-payload, the fluid head can have 4 pipette attachment nozzles located on 24 mm centers. Exemplary pipette tips that the pipette nozzles can capture without leakage include Biorobotix tips PN23500048 (50 μ L), PN23500049 (1.75 μ L), and PN23500046 (1 ml). The Z payload can incorporate a stepper actuated stripper plate capable of removing pipette tips (e.g., the pipette tips described above). The system can include a pump and manifold system that includes software controlled aspiration, dispensing, and venting of individual fluid volumes within each of the four individual tips and simultaneous dispensing and venting on all tips. The pump and manifold system can have an accuracy and precision of about ± 2 μ L per tip for volumes that are less than 20 μ L and about $\pm 10\%$ for volumes greater than or equal to 20 μ L (e.g., when aspirating or dispensing in individual tips). The total pump stroke volume can be greater than about 8 μ L and less than about 1250 μ L. The minimum aspirate and dispense speed can be about 10 μ L/sec to about 300 μ L/sec. The centroid of the bottom-most face of each pipette tip can be axially aligned with the nozzle centroid of the pipette nozzles within 0.2 mm. The bottom-most pipette tip faces can be co-planar within 0.2 mm. The Z-payload can incorporate a Z axis force sensor capable of feedback to software for applied forces of between about 0 and 4 lbs. The Z-payload can incorporate a downward facing barcode reader capable of reading the system barcodes as described elsewhere herein.

Referring now to racks included in the system, disposable reagent strips (e.g., oriented orthogonally to the front of the instrument) can be contained in 2, 12-lane racks. The 12 reagent strips in a given rack can register and lock into the rack upon insertion by a user. The rack can contain an area for 12 sample lysis tubes (e.g., PN 23500043) and hold the tube bottoms co-planar, allowing the user to orient the bar code to face the rear of the instrument. Certain features,

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including those listed above, can allow the racks to be inserted and oriented in the instrument by a minimally trained user. Proper rack placement can be confirmed by feedback to the software. In some embodiments, the racks can be black and color fast (e.g., the color may not appreciably degrade with use or washing with a 10% bleach solution) and the rack material can be dimensionally stable within 0.1 mm over the operating temperature range of the system. The rack can be designed with provisions to allow the rack can be carried to and from the instrument and to minimize or eliminate the likelihood that the tubes held by the rack will spill when placed on a flat surface.

Referring now to the reader and PCR heater included in the system, the reader can allow for cartridge insertion and removal by, for example, a minimally trained user. The cartridge can remain seated in the reader during system operation. In some embodiments, the cartridge barcode may not be read properly by the barcode scanner if the cartridge is inserted incorrectly (e.g., upside down or backwards), thus the system can instruct a user to correctly reinsert the cartridge into the reader tray when the cartridge is inserted incorrectly. The reader drawer can repeatedly locate the cartridge, for loading by the pipette tips, within 0.5 mm. The reader can deliver the cartridge from the loading position into a react and detect position by means of an automated drawer mechanism under software control. The PCR lanes of the cartridge can be aligned, with both the optical system and heater, by the reader tray and drawer mechanism. The cartridge can contact the heaters evenly with about a 1 psi, or greater, average pressure in the areas of the PCR channels and the wax valves. Heater wire bonds can be protected from damage so as not to interfere with system motion. Registration from heater to cartridge and from cartridge to optical path centers can be within ± 0.010 inches. The reader can mechanically cycle a minimum of about 80,000 motions without failure.

Referring now to the one or more lysis heaters included in the system, the heaters for each of the 24 lysis stations can be individually software controlled. The lysis ramp times (e.g., the time that it takes for the water in a lysis tube to rise from a temperature of approximately 2.5° C. to a given temperature) can be less than 120 seconds for a rise to 50° C. and less than 300 seconds for a rise to 75° C. The lysis temperature (e.g., as measured in the water contained in a lysis tube) can be maintained, by the lysis heaters, within $\pm 3^\circ$ C. of the desired temperature. The accessible lysis temperature range can be from about 40° C. to about 82° C. Each of the lysis heaters may draw about 16 Watts or more of power when in operation. The lysis heater can be designed to maximize the thermal transfer to the lysis tube and also accommodate the tolerances of the parts. The lysis heaters can permit the lysis tubes to be in direct contact with the magnets (described in more detail herein). The lysis heaters may be adjustable in the horizontal plane during assembly and may not interfere with the installed covers of the system.

Referring now to magnets included in the system, the lysis and magnet related mechanisms can fit beneath the rack and may not interfere with rack insertion or registration. The magnets may be high-flux magnets (e.g., have about a 1,000 gauss, or greater, flux as measured within a given lysis tube) and be able to move a distance sufficient to achieve magnetic bead separation in one or more of the lysis tubes filled to a volume of 900 μ L. The magnets can be software-controllable at movement rates from about 1 mm/sec to about 25 mm/sec. The wiring, included as part of the heater and controller assemblies, can be contained and protected from potential spills (e.g., spills of the lysis tubes). The magnets

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can be located about 1.25 inches or greater from the bottom of the lysis tube when not in use and can be retained in such a manner as to maximize contact with the lysis tube while also preventing jamming.

In some embodiments, the system enclosure includes a semi-transparent lid (e.g., with opaque fixtures and/or hardware) in the front of the instrument to allow users to view instrument functions. The lid can include a company and/or product logo and a graspable handle (e.g., enabling the user to raise the lid). When closed, the lid can have an opening force no greater than 15 pounds (e.g., when measured tangential to door rotation at the center of the bottom edge of the handle) and can lock in the open (e.g., "up") position such that no more than about 5 lbs. of force (e.g., applied at the handle and tangential to door rotation) is required to overcome the handle lock and return the lid to the closed position. The lid can include two safety lid locks that are normally locked when power is not applied and can allow the system to monitor the state (e.g., open or closed) of the lid. The lid can be designed such the lid does not fall when between the open and closed positions. The enclosure can include a power switch located on the right side of the instrument. A power cord can protrude from the enclosure in such a way that positioning the instrument does not damage the cords or cause accidental disconnection. The enclosure can prevent the user from coming in contact with, for example, moving parts, high magnetic fields, live electrical connections, and the like. The enclosure can include four supporting feet, located on the underside of the enclosure, to provide a clearance of about 0.75 inches or more between the underside of the enclosure and the table top. The enclosure can include a recessed area with access to external accessory connections such as the display port, the Ethernet port, the 4 USB ports, and the like.

Referring now to the cooling sub-system included in the PCR system, an air intake can be provided in the front of the unit and an air exhaust can be provided in the rear portion of the top of the unit. Intake air can pass through the air intake and through a filter element (e.g., a removable and washable filter element). The cooling sub-system can maintain an interior air temperature (e.g., the temperature as is measured at the surface of the reagent strips, such as the reagent strips numbered 1, 12, and 24, at the surface of the PCR cartridges, and the like) about 10° C. higher, or less, than the ambient air temperature. The cooling subsystem can maintain the internal air temperature at or below about 32° C. One or more cooling fans included as part of the cooling subsystem may require about 5.7 Watts, or less, of power per fan.

In some embodiments, the system can include covers on internal subassemblies (with the exception of the gantry). The covers can be cleanable with a 10% bleach solution applied with a soft cloth without significant degradation. The covers can supply a safety barrier between a user and the electronic and moving mechanical assemblies included in the system. The covers on the internal subassemblies can be designed to maximize cooling of the internal subassemblies by maximizing airflow under the covers and minimizing airflow above the covers. The covers can be removable by a service technician and can match the color and texture of the enclosures.

In some embodiments, the system can be designed to operate within a temperature range of about 15° C. to about 30° C. and in a non-condensing relative humidity range (e.g., about 15% to about 80% relative humidity). The analyzer can be designed to perform without damage after exposure to storage at no less than -20° C. for 24 hours or

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less, storage at no greater than 60° C. for 24 hours or less, and/or storage at about 50,000 feet or less (e.g., 3.4 inches of Hg) for 24 hours or less. The system can be designed with provisions to prevent motions that could damage the instrument during shipping. It can conform to the shipping standards set forth in ASTM D 4169-05, DC 12 and can be designed to allow the baseplate to be securely mounted to a shipping pallet. The racks and the enclosure of the instrument are designed not to degrade or be damaged by daily cleaning with a 10% bleach solution. The power to subassemblies of the system can be supplied by internal power supplies. Exemplary power supplies can receive, as input, about 1590 watts at about 90 to about 264 Vac at between about 47 and about 63 Hz and supply about 1250 watts of output to the subassemblies.

In some embodiments, the system can include a power switch (e.g., a rocker-type switch), located on the right side of the instrument, one or more interface components, and/or one or more interface ports. For example, the system can include an LCD display monitor that is 15 inches, has 1280x1024 pixel resolution and 16-bit color. The system can also include other display monitors such as ones with increased size, resolution, and/or color depth. The LCD display can be connected to the system via a VGA connection. The system can include a white, 2 button USB mouse, a white USB keyboard, a black SIT power cable, and an un-interruptible power supply, with feedback through USB. The system can also include a USB color printer, 2 USB cables (e.g., one for the printer and one for the UPS). The system can include exemplary interface ports, such as, 4 USB ports (e.g., to connect to a pointing device, printer, keyboard, UPS, LIS), 1 VGA port (e.g., for connection to the LCD display), and 1 Ethernet port (e.g., for PC connectivity) located on the left side of the enclosure. An IEC/EN 60320-11C14 power port can be included on the right side of the enclosure.

In some embodiments, the system can include features directed at increasing the safety of a user. For example, door interlocks can be included to prevent user access while the gantry is in motion and/or while other non-interruptible processes are underway. The system can be designed to minimize or eliminate the presence of user-accessible dangerous corners and/or edges on the instrument and designed such that metal parts are properly electrically grounded. Sheet metal or plastic covers can be included over mechanical and electrical components as necessary to protect a user from moving parts and/or live electrical parts and to protect the electronics and motors included in the system from, for example, spills.

Example 19: Exemplary Optics

Described herein are exemplary specifications related to the design of optics used in a PCR Analyzer and/or System. Additional information related to the PCR System is described elsewhere herein. The optical detection system included in the PCR System can be a 12-lane two-color detection system for monitoring real-time PCR fluorescence from a 12-lane microfluidic PCR cartridge. The system can include excitation lights (e.g., blue and amber LED light sources), one or more band pass filters, and one or more focusing lenses. The emitted fluorescence light from the PCR reactor (e.g., included in the microfluidic cartridge) is captured through a pathway into a focusing lens, through a filter, and onto a photodiode. Included in the system, for each PCR lane, are dedicated, fixed individual optical elements for each of the two colors interrogated.

In some embodiments, the limit of detection is 20 DNA copies per reaction of input PCR reaction mix with a minimum signal to base value of 1.15. The 2 color fluorescence system can be used with, for example, FAM (or equivalent) and Cal Red (or equivalent). The system can have the ability to collect fluorescence data in about 100 ms to about 600 ms at the maximum rate of one data point every about two seconds. When collecting data from a PCR lane, LEDs in adjacent lanes increase the signal in the lane being sampled by less than about 1% (e.g., 0.5%). The noise of the detection can be less than about 1% of the maximum signal. The lane-to-lane fluorescence variability with a fluorescence standard (e.g., part #14000009) can be within Cv of 30% for both FAM and Cal Red, when measured using the dark-current-corrected-fluorescence-slope. The average dark current-corrected-fluorescence-slope for the optical block with 12 lanes can be between about 30 mV to about 90 mV/(% blue LED power) for FAM using the fluorescence standard (Part #14000009). The average dark current-corrected-fluorescence-slope for the optical block with 12 lanes should be between about 75 mV to about 300 mV/(% amber LED power) for Cal Red using the standard fluorescence cartridge (Part #14000009). The average excitation power for each channel can be independently varied by software from about 5% to about 100%. There may be no source of light activated inside the reader to affect the fluorescence reading. In some embodiments, turning room lights on or off does not affect the optical readings.

In some embodiments, the system can include an optical block with 12 repeats of 2-color fluorescence detection units at a pitch of about 8 mm. The optical detection block can be positioned on top of the microfluidic cartridge, with excitation and emission travelling through the PCR windows of the microfluidic cartridge. The apertures of the optical block can align with the PCR reactor within about ± 200 microns. An optical electronics board containing the LEDs and Photodetectors can be mated flush with the top of the optics block with each of the photodetectors recessed into the bores of its corresponding optical lane. When the microfluidic cartridge is installed in the system, the optical block can be used to deliver a force of about 20 to about 30 lbs. over the active area of the microfluidic cartridge with an average pressure of at least about 1 psi.

The optical block can be made of aluminum and surfaces present in the optical path lengths can be anodized black, for example, to minimize auto-fluorescence as well as light scattering. An aperture plate having 12 slits, each slit about 10 mm in length and 1 mm wide, can be used, for example, to limit the size of the excitation light spots as well as reduce background fluorescence. The thickness of the optics block can be about 1.135 \pm 0.005 inches. The bottom surface of the optics block can be planar within ± 1 mil to provide uniform pressure over the micro fluidic cartridge. The apertures should be kept clean and free of debris during manufacturing of the optics block and assembly of the optics block into the system.

In some embodiments, the system can include excitation optics with an angle of excitation path equal to 55 \pm 0.5 inches with respect to normal of the PCR cartridge surface. One exemplary arrangement of optical elements in the excitation path, in order, is LED, lens, filter, aperture, and PCR sample. The system can use a Plano-convex excitation lens (e.g., PCX, 6 \times 9, MgF2TS) oriented with the flat side toward the PCR sample. Included in the optics are one or more excitation paths with tapers that can be designed such that the lens and filter can be placed inside the bore to provide a light spot bigger than the aperture plate. The

location of the LED and the sample can be fixed as the design can include a fixed available optical block thickness. The location of the lens and the filter can be determined to provide a excitation spot size of about 6 mm along the length of a PCR lane. The excitation optics can include an LED such as Luxeon Part # LXX2-PB 14-NO0 (e.g., for FAM excitation) that includes a center wavelength of about 470 nm (blue) with a half band width of about 75 nanometers, or less (e.g., for FAM excitation). The excitation optics can also include an LED such as Luxeon Part # LXX2-PL12-Q00 (e.g., for Cal Red excitation) that includes a center wavelength of 575 nm (amber) with a half band width of about 75 nanometers, or less (e.g., for Cal Red excitation). The LEDs used in the excitation optics can remain stable for about 5 years or more or about 10,000 cycles.

The system can include emission optics with an angle of emission path equal to about 15 \pm 0.5 inches with respect to normal of the PCR cartridge surface. One exemplary arrangement of optical elements in the emission path, in order, is PCR sample, aperture, filter, lens, and photodetector. The emission lens can be plano-convex (e.g., PCX, 6 \times 6 MgF2TS) with the flat side toward the photodetectors. The emission optics can include one or more bores, for the emission path, with tapers that can be designed so as to maximize detected light while enabling snug placement of the filters and lenses. The location of the photodetectors with respect to the sample can be fixed as the design can include a fixed available optical block thickness. The location of the lens and the filter can be determined so as to provide an emission spot size of 6 mm along the length of a PCR lane. An exemplary photodetector that can be used in the emission optics is the Hamamatsu Silicon Photodetector with Lens, S2386-18L.

In some embodiments, the system can include one or more filters with diameters of about 6.0 \pm 0.1 mm, thicknesses of about 6.0 \pm 0.1 mm, clear apertures with diameters of less than or equal to about 4 mm. The filters can include a blackened edge treatment performed prior to placement in a mounting ring. If present, the mounting ring can be metal and anodized black. The filters can be manufactured from optical glass with a surface quality that complies with F/F per Mil-C-48497A, an AOI of about 0 deg, a $\frac{1}{2}$ cone AOI of about +8 deg, and can be humidity and temperature stable within the recommend operating range of the system. An exemplary filter can be obtained from Omega Optical Brattleboro, Vt. 05301.

The system can include one or more FITC Exciter Filters (e.g., PN 14000001) with an Omega part number 481 AF30-RED-EXC (e.g., drawing #2006662) used, for example, in FAM excitation. These filters can have a cut-on wavelength of about 466 \pm 4 nm and a cut-off wavelength of about 496 \pm 4 nm. The transmission of filters of this type can be greater than or equal to about 65% of peak. These filters can have a blocking efficiency of greater than or equal to OD4 for wavelengths of ultraviolet to about 439 nm, of greater than or equal to OD4 for wavelengths of about 651 nm to about 1000 nm, of greater than or equal to OD5 for wavelengths of about 501 nm to about 650 nm, and of greater than or equal to OD8, in theory, for wavelengths of about 503 nm to about 580 nm.

The system can include one or more Amber Exciter Filters (e.g., PN 14000002) with a part number 582AF25-RED-EXC (e.g., drawing #2006664) used, for example, in Cal Red excitation. These filters can have a cut-on wavelength of about 569 \pm 5 nm and a cut-off wavelength of about 594 \pm 0 \pm 5 nm. The transmission of filters of this type can be greater than or equal to about 70% of peak. These filters can

have a blocking efficiency of greater than or equal to OD8, in theory, for wavelengths of about 600 nm to about 700 nm.

The system can include one or more FITC Emitter Filters (e.g., PN 14000005) with a part number 534AF40-RED-EM (e.g., drawing #2006663) used, for example, in FAM emission. These filters can have a cut-on wavelength of 514+/-2 nm and a cut-off wavelength of 554+/-5 nm. The transmission of filters of this type can be greater than or equal to about 70% of peak. These filters can have a blocking efficiency of greater than or equal to OD5 for wavelengths from ultraviolet to about 507 nm, of greater than or equal to OD8, in theory, from about 400 nm to about 504 nm, and of greater than or equal to OD4 avg. from about 593 nm to about 765 nm.

The system can include one or more Amber Emitter Filters (e.g., PN 14000006) with a part number 627AF30-RED-EM (e.g., drawing #2006665) used, for example, in Cal Red emission. These filters can have a cut-on wavelength of 612+5/-0 nm and a cut-off wavelength of 642+/-5 nm. The transmission of filters of this type can be greater than or equal to about 70% of peak. These filters can have a blocking efficiency of greater than or equal to OD5 for wavelengths from ultraviolet to about 605 nm, of greater than or equal to OD8, in theory, from about 550 nm to about 600 nm, and of greater than or equal to OD5 avg. from about 667 nm to about 900 nm.

Example 20: Exemplary 3-Layer Cartridge

Described herein are exemplary specifications used to design and assemble the microfluidic cartridge as well as exemplary instructions on the use of the cartridge in, for example, the system described herein. In some embodiments, the cartridge can have a maximum limit of detection equal to 20 copies per reaction volume (e.g., 20 copies/4µl), with a target detection of 10 copies per reaction volume. The cartridge can perform 45 reaction cycles in 40 minutes or less (e.g., 45 cycles in 40 minutes, 45 cycles in 20 minutes, 45 cycles in 15 minutes, or the like). The cartridge can utilize two color detection using, for example, the FAM (or equivalent) and CAL. RED (or equivalent) fluorescent dyes. Results obtained using the cartridge have been compared with the results obtained using standard real-time PCR instruments.

In some embodiments, the Cartridge can be a one-time use, disposable cartridge that can be disposed of according to typical laboratory procedures. The cartridge can be 4.375 inches long and 2.800 inches wide, with a thickness of 0.094+/-0.005 inches. The cartridge can include features that allow the cartridge to interface with, for example, the system described herein. Exemplary interfacing features include PCR channel walls and the top of the micro-substrate over the PCR channel that are well polished (SPI A1/A2/A3), enabling easy transfer of excitation and emission light between the PCR reactor (e.g., contained in the cartridge) and the detection system (e.g., the analyzer). The cartridge can include a thermal interface, located on the bottom of the cartridge, for interfacing with the analyzer. The thermal interface can have a thin laminate (e.g., less than 150 microns thick, 100 microns thick, or the like) to encourage heat transfer from the heater wafer to, for example, the PCR channels of the cartridge.

The cartridge can include one or more mechanical interfaces with, for example, the analyzer. For example, the cartridge can have a notch in one or more of the corners that can male with a corresponding shape on the heater module of the analyzer. The notch and corresponding shape can

enable the cartridge to be placed only one way in the tray of, for example, the system described herein. In some embodiments, the cartridge has a single notch in one of the corners, with the remaining three corners having a minimum radius of 1 mm to facilitate placement of the cartridge in the analyzer. During use (e.g., when placed in a system described herein and performing a function such as PCR), the cartridge can be pressed, on one side, by the optics block, against the heater wafer (positioned against the opposite side), with a pressure of about 1 psi or greater (e.g., 0.99 psi, 1.2 psi, or the like). When located in the tray of the analyzer, the cartridge can have an alignment slop of +/-200 microns to enable a user to easily place and remove the cartridge from the analyzer tray. The cartridge can have two ledges, that are each 1 mm wide and located along the two long edges of the cartridge, to enable the heating surface to extend below the datum of the tray.

In some embodiments, the cartridge can have the following functional specifications. The cartridge can include an inlet hole that is, for example, cone-shaped with a height of 1 mm from the top surface of the cartridge. The cone can have an inner diameter of 3 mm at the top of the cone and can taper down to a diameter that matches the width of a microchannel (e.g., an inlet channel) that the inlet cone is fluidly connected to. The inlet channel can fluidly connect the inlet hole to a PCR reactor that has an interior volume of, for example, about 4.25 µl to 4.75 µl (e.g., 4.22 µl, 4.5 µl, 4.75 µl, or the like). An outlet microfluidic channel can fluidly connect the PCR reactor to an overflow chamber. The cartridge can also include an outlet vent hole.

The input PCR sample (e.g., a reaction mixture) can be between about 6.0 and 7.0 µl per PCR lane (e.g., 5.9 µl per lane, 6.4 µl per lane, 7.1 µl per lane, or the like) and can be introduced into the cartridge through the inlet hole by, for example, a pipette. The reaction mixture can be transported, via the inlet channel, to the PCR reactor where the reaction mixture can be isolated (e.g., sealed off by valves) to prevent evaporation or movement of the reaction mixture during thermocycling. Once the mixture is sealed inside the chamber, the analyzer can initiate multiplexed real-time PCR on some or all of the reaction mixture (e.g., 4.5 µl, an amount of fluid equal to the inner volume of the reaction chamber, or the like).

The microfluidic substrate of the cartridge can include one or more of the following specifications. The material of the microsubstrate can be optically clear (e.g., have about 90% or greater optical transmission, be 3 mm thick, comply with ASTM D1003, and the like), have auto-fluorescence that is less than that emitted by 2 mm thick ZEONOR 1420R, and have a refractive index of about 1.53 (ASTM D542). The material of the microsubstrate can be amenable to the injection molding of features required for the microfluidic network of the cartridge. The material is preferably compatible with all PCR agents and can withstand temperatures of up to about 130° C. for about 5 minutes or more without yielding or melting. The cartridge can include fiducials, recognizable by HandyLab manufacturing equipment, located in one or more (preferably two) of the corners of the substrate. The cartridge can include fluidic components (e.g., microchannels, valves, end vents, reagent inlet holes, reaction chambers, and the like) necessary to perform the functions of the cartridge (e.g., PCR).

Additional features of the substrate material can include one or more of the following. Minimum clearances of about 1 mm can be designed between functional features to ensure sealing success (e.g., to the analyzer), and to allow simplified fixturing during assembly. The cartridge can include

dogbones under small fluid path ends to, for example, increase mold life. The bottom of the micro tool surface can be roughened (e.g., by vapor hone, EDM, or the like). The substrate material can be capable of adhesion by a label.

In some embodiments, the sealing tape used in the cartridge can include one or more of the following specifications. Laminate can be easily applied to the bottom of the microfluidic substrate. Material of the laminate is preferably pin-hole free. The material and adhesive is preferably compatible with the PCR reaction chemistries. The laminate material and glue used should not auto-fluoresce. The material can withstand up to 130° C. for 5 minutes without losing adhesion, yielding, melting, or causing undue stresses on the cartridge. Bubbles should not form in the adhesive layer upon heating (e.g., to 130° C. for 5 minutes) after application to the microsubstrate. The laminate should be less than 5 mils thick to, for example, enable rapid heat transfer.

The high temperature wax included in the cartridge can have the following characteristics. The wax should have a melt point of about 90+/-3° C. (e.g., 87° C., 90° C., 93.1° C., or the like), be biocompatible with PCR reactions, have wettability with microsubstrate material, and have a melt viscosity range, for example, of about Viscosity at 100° C.=20 mm²/s and Hardness at 25° C.=8 dmm. The main label of the cartridge can have the following characteristics. It can have a thickness of 2-4 mils, have suitable bondability to micro features and seal around the valves, include cuts for one or more PCR windows, and a tab (free from adhesive) for aiding in removal of the cartridge from the analyzer. The main label can also have abrasion resistance on the top surface, and be printable. The main label can have an upper and lower alignment pattern for the label to completely cover the valve holes for proper operation of the valves.

The cartridge can include a barcode label applied to the top of the cartridge that is readable by a barcode reader (e.g., the barcode reader included in the analyzer) while the cartridge is installed in the analyzer. The barcode label can include the product name, lot #, expiration date, bar code (2D) and may be printed on. In addition, or in the alternative, a barcode may be applied directly to the main cartridge label using a laser or inkjet type printer.

The packaging that the cartridge is included in can include one or more of the following: package label, carton, carton label, and/or operating instructions. The packaging can be printed on or label attachable, placed inside of a plastic bag, shrink/stretch wrap bag, or the like, and can be slacked in groups of 24. The cartridge bagging without a critical seal should be kept free from dust contamination.

The cartridge can include one or more valves (e.g., temperature controlled, wax-containing valves) for starting, stopping, and/or controlling the flow of material inside the cartridge. The wax contained in the valves can be free of trapped air bubbles that have a diameter greater than half the width of the valve channel. The valve channel can have an air pocket. The wax may not intrude into the fluid path prior to activation. The wax can be filled to the start of the flare to the fluid path.

The cartridge can include micro channels and holes such that the holes are of a size and shape to enable easy, leak-free interfacing with a 175 µl pipette tip. In some examples, the holes size is between about 200 µm and about 4000 µm in diameter. The microchannels can be between about 50 µm and about 1500 µm wide and between about 50 µm and 1000 µm high.

The cartridge can include valves for controlling the flow of fluid within the cartridge (e.g., through the microchannels, reactor chambers, and the like). The valve edges, steps,

and general geometry can be designed to encourage exact flow and/or stoppage required during wax load. The valve geometry can be designed to accommodate limitations of wax dispensing equipment (e.g., +/-25% of 75 nL volume). In some embodiments, step down air chambers on the valves are funnel shaped to aid wax loading and the remaining geometry diminishes from the bottom of the funnel to the end point where the wax stops. The path where the valves are to flow into and block, during use, can be narrow enough (e.g., 150-200 microns wide and deep) and have enough length to effectively seal when the valves are activated during use. The valve wax temperature can be about 90° C. When in use to block a portion of a microchannel, the valves can seal to prevent evaporation of fluid and/or physical migration of fluid from the PCR reactor during thermocycling.

The cartridge can include one or more PCR regions for performing PCR on a sample. The channel in the PCR region (e.g., PCR reactor) can be designed such that the temperature of the contents of the channel remain uniformly within about 1° C. of the anneal temperature. The channel walls can have a polish of SPI A1/A2/A3.

In some embodiments, the cartridge is designed to be able to perform diagnostic tests within a temperature range of about 59° F. to about 86° F. (about 15° C. to about 30° C.) and a humidity range of about 15% relative humidity to about 80% relative humidity. The cartridge is designed to be safe and functional when used indoors, used at an altitude of 2000 m or less, and used under non-condensing humidity conditions (e.g., maximum relative humidity of 80% for temperatures up to 31° C. decreasing linearly to 50% relative humidity at 40° C.).

In use, PCR product produced in the cartridge can remain in the used cartridge to, for example, minimize the likelihood of cross contamination. The cartridge can be designed such that a 4 foot drop of the cartridge, while in its packaging, will not damage the cartridge. The cartridge is designed to perform without damage after exposure to the following conditions. The cartridge should be stored at 4° C. to 40° C. for the rated shelf life. Exposure to temperatures between -20° C. and 4° C. or 40° C. and 60° C. should occur for no longer than 24 hours. The cartridge can withstand air pressure changes typical of air transport.

The cartridge can be labeled with the following information (e.g., to identify the cartridge, comply with regulations, and the like). The label can contain a "Research Use Only" label, if applicable, and a CE mark, if applicable. The label can contain the company name and logo (e.g., Handylab®), a part number (e.g., 55000009), a part name (12x Cartridge-nonvented), a lot number (e.g., LOT 123456), an expiration date (e.g., June 2015), space for writing, a barcode according to barcode specifications (described elsewhere), and/or "Handylab, Inc., Ann Arbor, Mich. 48108 USA".

The cartridge can be included in a carton that can contain information such as, a part number (e.g., 55000009), a part name (12x Cartridge-nonvented), a quantity (e.g., 24), a lot number (e.g., LOT 123456), an expiration date (e.g., June 2015), an optional UPC code, "Manufactured by Handylab, Inc. Ann Arbor, Mich. 48108 USA", a carton label to state storage limits, a CE mark (if applicable), and/or an AR name and address.

The cartridge packaging can include paper wrap to secure multiple cartridges together and clean package fill to prevent damage, for example, from vibration. The cartridge shipping carton can include features such as, compliance to ASTM 6159, carton may be stored in any direction, refrigeration or fragile labeling of the carton may not be required, and

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additional cold packs may not be required. The shelf life of the cartridge is 12 months or more.

The cartridge can comply with IEC 61010 (NRTL tested) and an FDA listing may be required for clinical distribution. Cartridges used in a clinical lab device may meet all quality system requirements. Cartridges used for research only in a commercial device may meet all HandyLab quality system requirements. Cartridges for research use only (Alpha or Beta testing) may be design/manufacturing traceable to a DHR (manufacturing record).

The foregoing description is intended to illustrate various aspects of the present inventions. It is not intended that the examples presented herein limit the scope of the present inventions. The technology now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. A system for processing a plurality of nucleic acid-containing samples, the system comprising:

a first module configured to extract nucleic acids from the plurality of nucleic acid-containing samples, the first module comprising:

a plurality of sample tubes in the first module, each sample tube configured to accept a nucleic acid-containing sample of the plurality of nucleic-acid containing samples,

a plurality of process chambers in the first module, wherein a process chamber of the plurality of process chambers is spatially separate from, and corresponds to, a sample tube of the plurality of sample tubes, the plurality of process chambers maintained at a same height relative to one another in the first module,

a waste chamber in the first module, the waste chamber corresponding to a process chamber of the plurality of process chambers in the first module,

a magnetic separator configured to apply a magnetic force to at least one process chamber of the plurality of process chambers in the first module;

a heater assembly configured to heat at least one process chamber of the plurality of process chambers in the first module;

a second module configured to receive nucleic acids extracted from the plurality of nucleic acid-containing samples, the second module comprising:

a plurality of receptacles comprising PCR reagents, wherein a receptacle of the plurality of receptacles is configured to receive nucleic acid extracted from a sample of the plurality of nucleic acid-containing samples; and

a liquid dispenser configured to dispense or withdraw liquid from the plurality of sample tubes and dispense or withdraw liquid from the plurality of receptacles comprising PCR reagents.

2. The system of claim 1, wherein the liquid dispenser comprises one or more dispense heads configured to accept a pipette tip.

3. The system of claim 2, wherein the liquid dispenser comprises four dispense heads and the plurality of process chambers comprises twelve process chambers, each dispense head configured to dispense a plurality of magnetic binding particles and at least a portion of a sample of the plurality of nucleic acid-containing samples into one of the twelve process chambers in the first module.

4. The system of claim 1, further comprising a sample identification verifier configured to check an identity of each sample of the plurality of nucleic acid-containing samples,

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wherein the sample identification verifier is selected from the group consisting of an optical character reader, a bar code reader, and a radio frequency tag reader.

5. The system of claim 1, further comprising electronic circuitry configured to control operation of the magnetic separator, the heater assembly, and the liquid dispenser.

6. The system of claim 5, wherein the electronic circuitry is configured to cause the magnetic separator to apply a magnetic force to the plurality of process chambers in the first module.

7. The system of claim 5, wherein the electronic circuitry is configured to cause the heater assembly to apply heat to the plurality of process chamber in the first module.

8. The system of claim 5, wherein the electronic circuitry is configured to control motion of the liquid dispenser.

9. The system of claim 1, further comprising one or more processors and at least one input device coupled to the one or more processors, the at least one input device selected from the group consisting of: a keyboard, a touch-sensitive surface configured to accept input from a stylus or a user's finger, a microphone, a track-pad, a retinal scanner, a fingerprint reader, a holographically projected interface, and a mouse.

10. The system of claim 9, further comprising a communication interface coupled to the one or more processors, the communication interface being selected from the group consisting of: a serial connection, a parallel connection, a wireless network connection, a wired network connection, and one or more USB ports.

11. The system of claim 10, further comprising a data storage medium configured to receive data from the one or more processors, the at least one input device, and the communication interface, the storage medium being selected from the group consisting of: a hard disk drive, an optical disk drive, a flash-card, a USB-drive, and a CD-Rom.

12. The system of claim 11, further comprising at least one output device coupled to the one or more processors, the at least one output device being selected from a visual display, a printer, a holographic projection, and a speaker.

13. The system of claim 1, wherein the PCR reagents comprise a first lyophilized PCR reagent suitable for detecting a first analyte and a second lyophilized PCR reagent suitable for detecting a second analyte.

14. The system of claim 1, wherein the second module has more than one area for receiving nucleic acids extracted from the plurality of nucleic acid-containing samples.

15. The system of claim 14, wherein the one or more areas are cooled independently of one another.

16. The system of claim 1, further comprising a second plurality of process chambers in the first module.

17. The system of claim 1, further configured to simultaneously amplify the nucleic acid extracted from the plurality of nucleic acid-containing samples.

18. The system of claim 17, wherein the number of nucleic acid-containing samples is twelve.

19. The system of claim 17, further comprising an optical detection system configured to independently detect a plurality of fluorescent dyes at a plurality of different locations, wherein each fluorescent dye binds to a fluorescent polynucleotide probe or a fragment thereof.

20. The system of claim 19, wherein the optical detection system selectively emits light in an absorption band of the plurality of fluorescent dyes and selectively detects light in an emission band of the plurality of fluorescent dyes.

21. The system of claim 19, configured to carry out extraction, amplification, and detection of the plurality of nucleic acid-containing samples in less than an hour.

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22. The system of claim 1, further comprising a heater substrate comprising at least one heat source configured to apply heat at one or more selected times in order to apply thermocycling operations sufficient to amplify the nucleic acid extracted from the plurality of nucleic acid-containing 5 samples.

23. The system of claim 22, wherein the at least one heat source is configured to maintain a negligible temperature gradient across a reaction zone during the thermocycling operations, the reaction zone configured to receive the 10 nucleic acid extracted from one of the plurality of nucleic acid-containing samples.

24. The system of claim 23, wherein the at least one heat source is configured to maintain a negligible temperature gradient across each of a plurality of reaction zones during 15 the thermocycling operations, each reaction zone configured to receive the nucleic acid extracted from one of the plurality of nucleic acid-containing samples.

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